Vol. 21 No. 5

May, 1955

# THE BOTANICAL REVIEW

Interpreting Botanical Progress

Studies of Mineral Nutrition by Use of Tracers.
ORLIN BIDDULPH 251

FUTURE CONTENTS
ON INSIDE BACK COVER

Published Monthly except August and September, with
a combined January–February–March issue,
at Box 749, Lancaster, Pa.

Entered as second-class mail matter February 4, 1935, at the Post Office at
Lancaster, Pennsylvania, under the Act of March 3, 1879.

# THE BOTANICAL REVIEW

*Founded in 1935 by*
HENRY ALLAN GLEASON and EDMUND H. FULLING
*Managed, edited and published at*
*The New York Botanical Garden*
*by*
EDMUND H. FULLING

---

*Advisory Editors*

PROF. CHESTER A. ARNOLD
University of Michigan
PROF. MYRON P. BACKUS
University of Wisconsin
PROF. HERBERT C. HANSON
Catholic University of America
PROF. M. M. RHOADES
University of Illinois
PROF. GILBERT SMITH
Stanford University
DR. RUSSELL B. STEVENS
National Research Council
PROF. S. F. TRELEASE
Columbia University
PROF. CONWAY ZIRKLE
University of Pennsylvania

THE BOTANICAL REVIEW is published monthly except August and September, with a combined January-February-March issue. Subscription rate for all countries is $6.00; single copies 60¢. All subscriptions are payable in advance at par in Lancaster, Pa., or New York. Checks should be made payable to THE BOTANICAL REVIEW.

Articles are obtained primarily by invitation. Unsolicited manuscripts are also welcome. In all cases, however, the editor reserves the right to accept, reject or suggest revisions in submitted material. All manuscripts should be on double-space typewritten pages, with references and citations in accordance with the customary style of THE BOTANICAL REVIEW.

Missing numbers can not be supplied unless notice is received within two months after appearance of issues concerned.

All correspondence should be addressed to THE BOTANICAL REVIEW, at Box 749, Lancaster, Pa., or Fordham P. O., New York, N. Y.

THE BOTANICAL REVIEW

VOL. XXI MAY, 1955 No. 5

# STUDIES OF MINERAL NUTRITION BY USE OF TRACERS

ORLIN BIDDULPH

*State College of Washington, Pullman, Washington*

Introduction ..... 251
Procedures ..... 256
Direct Counting ..... 258
Autoradiography ..... 260
Absorption ..... 262
Translocation ..... 271
Metabolism ..... 275
Calcium ..... 275
Copper ..... 275
Iodine ..... 276
Iron ..... 276
Manganese ..... 276
Molybdenum ..... 277
Phosphorus ..... 277
Sulfur ..... 280
Sodium ..... 281
Zinc ..... 282
Radiation Injury ..... 282
Bibliography ..... 286

## INTRODUCTION

A completely satisfactory arrangement for grouping papers into topics for this review has not occurred to the writer; consequently the system employed may seem rather arbitrary. An attempt has been made to show the relevance of each paper to the solution of particular problems, wherever possible, hence the headings "absorption" and "translocation"; but when the topic of metabolism is encountered it will become obvious that only disjunct contributions are offered. In this area plant physiologists can show progress with only two tracer elements, i.e., phosphorus and sulfur, and even here metabolic patterns are hardly mentioned. Most of the work is still best described as "geographic tracing" rather than as true "metabolic tracing". The other headings, "introduction", "procedures" and "radiation injury", are self-explanatory. The writer's justification for the omission of review type articles, of

which there were countless numbers in the early days of tracers, is that the useful parts of them are now adequately covered in textbooks.

The basic requirement of a tracer is that it be "chemically and physically exactly equivalent to the substance it represents or displaces and that it in no appreciable way affects the system differently from its normal counterpart" (Siri, 1949). Many of the radioactive tracers successfully fulfill these requirements, but this condition is not altogether satisfied with $H^3$ as a tracer for hydrogen or $C^{14}$ as a tracer for carbon. Beyond this atomic weight the difference between the behavior of the radioactive isotope and the naturally occurring isotope is so small as to be negligible. For instance, the process of diffusion and the rate of reaction will be influenced by the mass of the isotopes quantitatively as $\frac{1}{\sqrt{\text{density}}}$; hence a rather wide difference in masses is required before the effect is large enough to be measurable. Heavy water (deuterium) is known to have a toxic effect and hence behaves differently from ordinary water (Barnes and Jahn, 1934). A differential effect in favor of one isotope over another has been noted for carbon and oxygen isotopes (Kamen, 1946), but it is with tritium that the greatest isotopic effect is to be expected.

The processes of diffusion and metabolism, in a single cycle through a plant, do not affect an isotopic separation of any magnitude for elements of higher atomic weights. Mullins and Zerahn (1948) have examined the $K^{40}$ content of potassium from animal, vegetable and mineral sources, and compared it with reagent KCl. No difference in $K^{40}$ content within $\pm 0.5\%$ could be demonstrated. A number of cases were cited in the above reference wherein an isotopic separation had been reported (for *Valonia* and *Nitella;* see Jacques, 1940).

It has been shown (Vinogradov and Teis, 1941) that $H_2O^{16}$ and $H_2O^{18}$ are used indiscriminately in photosynthesis and that the $O_2$ produced in photosynthesis comes from the water (Rubens et al., 1941). Therefore neither isotope of oxygen is favored in oxygen production; however, the heavier isotope of oxygen may react more slowly in respiration, as has been suggested by Rabinowitch (1945) to offer a probable explanation for the observation that atmospheric oxygen is about $7.5 \times 10^{-5}$ atomic weight units heavier

than the oxygen in water of the oceans (Swartout and Dole, 1939). Cycling through many plants during many ages may have affected an appreciable isotopic separation.

The $C^{12}/C^{13}$ ratio of 105 plants representing all major plant groups has been determined by Weckman (1952). With the possible exception of Gymnosperms, there appeared no systematic differences between groups. The local carbon dioxide cycle, however, was responsible for some variations between individuals.

Radioactive tracers may also affect biological systems differently from normal elements in that they emit ionizing radiations which are responsible for the production of molecular fragments of protoplasmic constituents. However, this review will concern itself only with those cases where attempts have been made to establish a threshold involving the first injury to some physiological process. When radioisotopes are used in what is commonly called " tracer amounts ", the biological effects of the radiation are frequently disregarded because of the smallness of the effect. However, as more refinement is attained, it may be necessary to revise our concepts as to the threshold value wherein a radioactive tracer first alters a physiological process. (See Mackie et al., 1952.)

According to Hevesy (1948*a*), by whom the first use of tracers was made, the historical background for this type of study dates from the attempts made in Rutherford's laboratory to separate the radium D embedded in the lead obtained in the separation of radium from pitchblend. Failure in 1911 and 1912 to effect a separation led Hevesy to find a use for the unseparated material. This he and Paneth, after similar failure, used to determine the solubility of lead salts, and later with Zechmeister (Hevesy and Zechmeister, 1920) to show that lead in lead salts did not interchange with lead ions in solution. Organic lead compounds showed the same absence of interchange. Hevesy (1923) then used this material to follow the uptake of lead by plants.

Later work has shown that neither labeled phosphorus (Hahn and Hevesy, 1940) nor sulfur (Tuck, 1939) interchanges when brought together with organic compounds present in organisms. This, however, is not true of hydrogen bound by oxygen or nitrogen (Reitz and Bonhoeffer, 1934). As it would have been impossible to advantageously use as a tracer any atoms which randomly exchanged positions with other atoms in organic combina-

tion, it was of fundamental importance to establish the fact that physical interchange did not occur.

With this basic fact established, it became practical to introduce marked atoms into any biological system which could be sufficiently isolated for study and to follow the normal course of reactions throughout the process. This technique allows discernment of the experimental atoms from those initially present in the organism and permits one to follow the " growth " of these particular atoms into or through any given system. With this method a new, or at least a much more dynamic, view of the " turnover " of atoms and molecules within living systems has resulted. The concept that the materials taken in do not pass through and become exhausted as wastes, but instead that they enter into the structure of the organism as they are metabolized, persist there for a characteristic period and then become discharged to make way for more recently acquired materials, is a basic concept of biology which has come to full realization by the use of tracers.

True, there were adherents to this view prior to the widespread use of tracers, but the concrete evidence for the extent of turnover and the quantitative expression of turnover rates, especially the exact mechanisms of turnover, have been acquired only recently. The realization that such a dynamic system exists is a basic contribution to the fundamental concepts of biology.

Among the advantages of the tracer methods is the ability to identify one's experimental atoms from all others and to follow them in their geographic wanderings or through their various chemical combinations. A similar advantage is the minuteness of the number of atoms that can be so followed. A few thousand atoms can easily be detected and quite accurately measured in a quantitative sense (DuBridge, 1938).

The disadvantages are related to the advantages. If such a few atoms can be detected, they can, if carelessly handled, contaminate samples and render determinations uncertain. A whole new standard of laboratory cleanliness has emerged and has attained a level of perfection, even above that employed in the handling of pathogenic organisms. The pathogenicity of an organism can be destroyed by heat—radioactivity is a physical property of the atom and as such remains unaltered, except by its characteristic natural decay. In the homely process of washing dishes one must be ac-

tively concerned with the physical removal and proper disposal of the last atom. Another disadvantage is the effect of the radioactive emanations on the processes being studied; this will be treated in a later section.

When considering a procedure involving radioactive isotopes as a possible means to the solution of a specific problem, one must bear in mind convenience, rapidity and accuracy; and in addition, consideration must be given to the danger of spillage and contamination. One is often intrigued by the apparent rapidity with which assays may be made on a prepared sample with little regard to the laborious procedure of obtaining the sample. In their preparation extreme care must be exercised in guarding against contamination between samples and in contamination of the laboratory in general which would render it difficult to continue work without contaminating future samples. Health precautions must also be realized. Finally, disposal of the radioactive material in a proper way oftentimes presents definite problems. All of these considerations should be fully appraised before one attempts a solution of a specific problem by the use of radioactive isotopes [1]. After a number of years experience, it is generally conceded by most workers that if an answer can be obtained in some other way than by tracer methods, the labor involved would not justify the latter. However, there are so many physiological problems for which a direct answer can be obtained only by utilization of marked atoms that this method should become a standard procedure in all physiological laboratories. Once the initial setup is in operation, the attack on specific problems becomes much simplified, and more problems can be justifiably attempted by this means. So many advances have been made by means of these procedures that it is no longer necessary to defend their use.

The amount of radioactive material which it is necessary to handle in tracer work presents no serious health hazard to an experienced user if reasonable caution is exercised. It is the opinion of the writer that improper handling of radioactive materials in

[1] A number of pamphlets on health precautions and procedures are available from the Technical Information Division, U.S.A.E.C., Oak Ridge, Tennessee. Two handbooks by the National Bureau of Standards, i.e., *Safe Handling of Radioactive Isotopes* (Handbook 42) and *Control and Removal of Radioactive Contamination in Laboratories* (Handbook 48) are available through the Superintendent of Documents, Washington 25, D. C., for 15 cents each.

tracer amounts will render measurements unreliable and the laboratory useless as a working place prior to the time that health considerations become a serious factor.

## PROCEDURES

Since the time of Becquerel (1896) it has been known that radioactive materials possess the important property of discharging electrified bodies. Rutherford (1899) found that this discharging action could be explained on the assumption that the gas was ionized by the passage of the radiations through it. Mme. Curie (1898) employed a suitable electroscope to measure the ionization produced by radioactive bodies. Based on these principles, but at a later date, the now familiar Geiger-Mueller (1928, 1929) counter was devised. These fundamental procedures have lately been employed together with elaborate recording circuits for the accurate measurement of radioactive substances.

The standard textbooks on radioactive tracers now serve as a general outline for the common procedures employed in biological studies (Hevesy, 1948*b;* Siri, 1949; Kamen, 1951). There are, however, several articles covering specialized techniques which may be worthy of mention here. Radin (1947, 1948), in a series of five articles, has presented a comprehensive treatise of methods, backgrounds for methods, and basic concepts. The general philosophy of tracer uses is presented. Cohn (1948) has given a discussion on the origin of contaminants in radioisotopes together with methods of detection and removal. Yankwich (1949) has covered errors, isotope dilution analysis, purity and isotope effects. Instrumentation has been discussed by Borkowski (1949) who included Geiger-Mueller counters, proportional counters, ionization chambers and electroscopes, and gave a comparison of counting rates for the different devices. Measurement techniques are reviewed by Kohman (1949) in a discussion of sample preparation, sample errors, instrument errors, absolute measurements and statistical considerations. A discussion of radioactive tracers in plant nutrition has been presented by Martin and Russell (1950). Their article covers the chemical and physical behavior of isotopes before disintegration, the effects of the new substances formed, and the effects of the radiation they emit. The general value of tracer

methods is also considered. Most of the above topics also receive attention in the standard texts.

There are instances wherein the use of tritium in marking water would seem desirable. The techniques for measuring tritium are somewhat more complex and involve the decomposition of water and determination of tritium as gaseous hydrogen. Several procedures have been outlined. Henriques and Margnetti (1946) describe a procedure based upon the insertion of hydrogen gas in a quartz ionization chamber attached to a Lauritsen electroscope. Robinson (1951) caused radioactive water to react with methyl Gringard reagent to give radioactive methane which was emitted in a proportional counting chamber. Libby presents procedures and theories for $C^{14}$, $S^{35}$, T and others (1947), and in later articles Grosse et al. (1951) and Wolfgang and Libby (1952) describe a procedure for determining tritium based on the decomposition of water by zinc and the introduction of the hydrogen sample into a Geiger-Mueller tube with ethylene and argon as counting gases. The writer has used this procedure to advantage where small bits of tissue are to be analyzed. There is some question as to the value of tritium as a marker for water. The isotope effect should be maximum here and render the behavior of radioactive water slightly different from normal water in biological systems. Before employing this method, one should consider carefully whether an isotope effect will be a disadvantage in the procedure being contemplated. Wherever metabolic water is concerned, the isotope effect is to be expected, as it also is in absorption, a process involving diffusion.

There are some instances wherein an " in vivo " assay of radioisotopes in plants is highly desirable. This would be particularly advantageous on expanded organs such as leaves or on stems where rates of movement are to be determined. Pace et al. (1948) have presented a design for a small Geiger-Mueller tube to be used with gamma emitters. Klechkovskii, Tselishcher and Evdokimora (1951) have used shielded counter tubes for determination of radioactivity in leaves, and Martin (1952) has developed a switching device in order that four G-M tubes could be successively fed into one scaling circuit. The assay of fresh tissue in place should also prove most valuable as a pilot method for determining the ap-

pearance of radioactive materials in tissues prior to an accurate analysis either by direct counting or by autoradiographic preparations.

DIRECT COUNTING. Direct bombardment of tissues in a pile is a possible way of inducing radioactivity in certain trace elements, i.e., those with a sufficiently large " cross section ", to readily effect neutron capture at the flux employed. Tobias and Dunn (1949) show a typical distribution of radioactivity obtained together with the decay characteristics.

Direct assay of radioactivity in solutions has appealed to a number of investigators, and several systems for conducting such analyses have been offered. It should be pointed out, however, that the majority of investigators are using counting procedures based on solid or gaseous samples. A dipping counter tube has been described by Bale et al. (1939). Smith and Cowie (1941) describe methods used in preparing standards and in determination of $C^{11}$ in liquid samples. McAuliffe (1949) describes a rapid method for $P^{32}$ determination in solutions which flow around the sensitive portion of the G-M counter, in which he claims an accuracy to one per cent. Schweitzer (1949) compares three methods of measuring radioactivity in solution: open solution counting allowed detection of the lowest energy particles; the enclosed solution method was the safest; and the dipping counter method was the least efficient. Freedman and Hume (1950) describe a method of covering liquid samples with lacquer so that they may be used with safety under the window of a G-M tube. Fluids containing radioactive elements may also be dropped from a micropipette onto filter paper which can be dried and cemented onto a metal disk for " counting " (Burch et al., 1950).

A mathematical treatment of the possibilities of using isotopes to determine the rate of a biological reaction has been prepared by Branson (1947). This is an area into which biologists should attempt an invasion. Fundamental treatments of the theory of rate of reaction can be found in physical chemistry literature.

Special care must be exercised in handling radiophosphorus because of its tendency to adsorb onto glassware. In order to avoid this difficulty, Hall and MacKenzie (1947) used dimethyldichlorosilane (a precursor of silicone) to coat their pipettes, graduated

cylinders, centrifuge tubes, etc. Several commercial preparations are available for this purpose. Several sets of glassware were coated, as there was a tendency for abrasion, dust and grease to cause the coated surface to lose its hydrophobic characteristics. Vessels of different kinds were also treated with butyl methacrylate.

In preparing samples for analysis, MacKenzie and Dean (1948) found it advantageous to convert phosphorus to $MgNH_4PO_4 \cdot 6 H_2O$. This material was filtered onto a suitable holder so that $P^{32}$ and total phosphorus could be determined on a single preparation. In addition, they offer a number of suggestions concerning the use of G-M counters and counting conditions.

The desirability of counting compressed plant material directly in order to avoid the lengthy chemical procedures necessary to obtain purified precipitates has led MacKenzie and Dean (1950) to develop a procedure of converting plant material into briquets and determining the activity of the compacted material directly. After standardization of their procedure, specific activity could be calculated from measured activity and a total phosphorus determination on a portion of the sample. This procedure is particularly desirable when a large number of crop plants must be surveyed in the shortest possible time.

The assay of radiosulfur presents some difficulties which are not serious with radiophosphorus. The former is frequently assayed from a preparation which is infinitely thick with respect to the path of the emerging electron in the sample (Hendricks et al., 1943), while the latter is more often " counted " as a " thin " preparation. Use of a " flow counter " for $S^{35}$ makes possible the use of thin samples if the amount of material necessary for the assay can be spread infinitely thin with respect to the path of the electron in the sample material.

It is sometimes desirable to introduce a radioactive isotope into plant material which is to be used in feeding studies involving animals. It is then desirable to know how to acquire the highest possible activity in the particular plant fraction to be used. This involves a knowledge of the absorption and translocation characteristics of the element in the plant in question. Jacobson (1948) describes a procedure for introducing radioactive phosphorus into the grain of growing corn. He found it advantageous to introduce the radioactive phosphorus into the nutrient solution when the

corn was in the early milk stage of kernel production. The mature corn grain then had the highest radioactivity of any part of the corn plant. Sufficient radioactivity in the grain was obtained for satisfactory feeding experiments with poultry. Using the same principle to a different end, White, Fried and Ohlrogge (1949) showed green manure containing $P^{32}$ to be as effective a source of phosphorus to plants as potassium dihydrogen phosphate on a phosphorus-deficient soil.

AUTORADIOGRAPHY. Although the fact that radioactive materials would affect a photographic plate was discovered by Becquerel in 1896 and the first autoradiographic studies were made as early as 1904 (London, 1904), the autoradiographic technique was not widely used until pile-produced isotopes became available. The whole tissue technique for the preparation of autoradiographs can be easily mastered by anyone who has some knowledge of dark-room procedures. The quality of the radiograph will depend upon a number of factors, namely, the energy of the particle emitted by the radioactive material (the lower energies resulting in the highest resolution), the degree of contact between the plant material and the film, the choice of film, and the choice of the proper exposure time. Care must be taken to protect the film from contacts which would cause it to blacken as a result of chemical reactions (Boyd and Board, 1949). No-screen x-ray film has been most widely used for this gross type of autoradiograph, since it combines speed with a maximum silver halide content. Rediske and Biddulph (1953) found it suitable for such weak emitters as $Fe^{55}$. A procedure for simultaneously preparing a number of autoradiographs has been described by Wittwer and Lundahl (1951).

The gross radiographic technique provides an expedient tool for the study of absorption, translocation and distribution of various elements in plants. Arnon, Stout and Sippos (1940) traced the metabolism of inorganic phosphates in the leaves and fruits of tomato and found that the younger the fruit the greater the capacity for phosphorus absorption, although fully ripe fruits still attached to the plant continued to accumulate small amounts of phosphorus in the pulp. Colwell (1942) applied radioactive phosphorus to squash leaves and studied its translocation to other parts of the plant by means of autoradiographs. Harrison, Thomas and Hill (1944) were the first to use sulfur autoradiographs and

showed the distribution of this element in wheat. In a particularly striking report Sayre (1952) made a systematic study of the differential accumulation of 14 radioactive elements in the leaves of corn plants. Zinc nutrition of the tomato vine grown from germination to maturity with all the zinc tagged by $Zn^{65}$ was studied by Stout et al. (1947). They found a greater portion of the zinc in seeds and conducting tissues than in the pulp of the fruit. They also noted that the abscission tissue on the stem of the fruit absorbed more zinc than the stem above or below this area. Grosse and Snyder (1947) have published autoradiographs of geranium leaves which had been exposed to an atmosphere containing $C^{14}$. Translocation studies of a number of elements in red kidney bean have been carried out by Biddulph (1951, 1953) and by Rediske and Biddulph (1953) who used iron and phosphorus to study absorption and translocation in this plant.

Out of this gross autoradiographic technique has grown a more refined or histological technique wherein an attempt is made to correlate the source of radiation with an exact locality or tissue within the plant. The number of difficulties encountered in this new method has resulted in many studies to obtain a more precise localization of the radioactive isotope with respect to the film. One of the major problems has been to develop a suitable emulsion —the ideal one having small grain size, a high and uniform silver bromide content and speed, a combination difficult to obtain. In addition, it has been necessary to develop extremely thin emulsions which can be closely applied to the tissue section and which have a selective response to the different types of radiation. Track emulsions have helped to alleviate the difficulty caused by " spread " of the activity in the emulsion due to the discharge of particles in all directions from the isotope.

There are other attendant difficulties in the preparation and mounting of tissue without loss of soluble tracer and in the incorporation into tissue without injury of enough isotope to give the large radiation intensities needed for proper exposure of the film. The only practical way to avoid loss of soluble tracer from tissue seems to be by freeze-drying (Holt, Cowing and Warren, 1949). Freeze-drying is also indicated when rapid decay of the isotope would make conventional tissue preparation impractical. At present this technique has been more extensively applied to animal

tissue than to plant tissue. According to Boyd and Levi (1950), the problem of getting images without incorporation of such large amounts of isotope into the tissue is partially solved by the newer $\beta$-track films.

The basic problem, that of getting a close correlation of image and section so that there is a precise localization of the radiation source, has been approached in a number of ways. The first method tried was to clamp the mounted section to the emulsion side of the photographic plate which was later developed. In this case the autoradiograph could not be observed in direct relationship to the histological section which produced it. To meet this difficulty Belanger and Leblond (1946) poured a melted emulsion over the section, and Pelc (1947) covered the section with a photographic emulsion stripped from a photographic plate. Some workers have mounted the histological section directly on the emulsion (Evans, 1947; Bourne, 1952; Russell, Sanders and Bishop, 1949; Endicott and Yagoda, 1947). Most recently Gomberg (1951) has gone back to the wet-celloidin photography of the 19th century and has evolved a process of emulsion formation over the histologic section mounted on a slide. The process is believed to extend to about one micron the resolution possible with weak beta emitters.

Microautoradiography has been much more extensively used with animal tissue than with plant tissue. Since the method is a new one, even these papers deal principally with techniques (Duggar and Moreland, 1953). Howard and Pelc (1951) have demonstrated the nuclear incorporation of $P^{32}$ in the roots of *Vicia faba* seedlings. They found indications that incorporation of phosphorus stops sometime before visible prophase and does not occur during the actual division. From their autoradiographs, Russell, Sanders and Bishop (1949) concluded that the concentration of phosphorus in young barley plants occurred especially in the apical meristem, the leaf primordia, the bases of the younger leaf sheaths and initials of adventitious roots.

The development of the phase microscope has made the study of unstained sections practicable. Removal of paraffin from the section and superimposition of a sensitive emulsion complete the tissue preparation. After exposure and development the combined tissue section and autoradiograph can be studied together.

## ABSORPTION

The literature on absorption problems was voluminous and the topic a lively one for investigation prior to the widespread use of "tracers". However, when applied they materially aided in a clear resolution of absorption problems into surface exchanges and metabolic uptake. Although the duality of the phenomenon was quite generally recognized prior to that time, the tracer method, by its ability to differentiate the experimenter's "probe" atoms from the cell's general atomic complex, facilitated in obtaining a clearer expression of the idea.

Radioactive lead was first used to attack permeability problems (Hevesy, 1923). By this means it was shown that lead atoms associated with roots remain replaceable by other lead atoms, hence are not in organic combination. It was shown (Lark-Horovitz, 1929) that lead ions do not readily enter the sap of the living cell but will penetrate dead cells readily. Radon, however, distributes itself evenly between cell sap and the bathing solution. Hevesy, Linderstrom-Lang and Nielson (1937) used phosphorus to determine the exchange between phosphorus atoms of yeast cells and those of the nutrient solution. They found that yeast did not exchange its phosphorus. Their views were that phosphorus was either present in organic combination and hence not exchangeable or that the cells in the mature state were impermeable to phosphate ions. Mazia (1940) summarized his views on the binding of ions by the cell's surface. He pictured the cell's surface as simultaneously a barrier and a reservoir for ions. The binding of ions in the surface, in general, prevented their penetration into the interior. There appeared, however, conditions under which the bound ions might be released into the interior and might set into motion far-reaching changes.

Spiegelman and Reiner (1942) presented a mathematical treatment of the problems of permeability, and as a result suggested that chemical forces, as distinguished from electrical, should provide a more fruitful approach to the problems of potassium and sodium balance within cells. Holm-Jensen et al. (1944) derived formulas for expressing diffusion constants in cm./hr. They showed that permeation resistance is mainly in the outer protoplasmic membrane; the inner is responsible for a few per cent at most. They hold that concentration differences within and without a cell are

maintained as a steady state phenomenon. Krogh (1946) reviewed the problems of permeability and exchange, and presented a method of distinguishing between active ion uptake and passive permeability. A formula for expressing permeability in centimeters per hour was included.

Brooks (1937, 1938*a*, 1938*b*, 1938*c*, 1939, 1940) undertook a study of selective accumulation in relation to permeability and was able to trace migration from the bathing solution across the cytoplasm to the vacuole. He visualized potassium as moving in combination with protein constituents. The initial stage of uptake was regarded as being an exchange reaction (for non-isotopic potassium). This was followed by a steady intake in some way related to metabolic processes. The first stage of uptake was later referred to as "induced accumulation" which appears to be an exchange phenomenon. This state is followed by "primary accumulation" which seems to be related to the metabolic accumulation later referred to by Hoagland (1940). The relationship of the exchange reactions to other substances was studied by Mullins and Brooks (1939) and Mullins (1941). The technique used in these studies was that of following the outgo of the labelled element from cells to the bathing solution, the bathing solution containing the elements which were tested for their effects on permeability. The rate of loss induced by the test elements followed the Hoffmeister series. Rubidium was the fastest and lithium the slowest in replacing potassium. For sodium, the Hoffmeister series was reversed. Very little loss occurred to distilled water. They furthermore showed that recently absorbed ions were more easily replaced than those acquired earlier. This was due to the probable absorption of radioactive tracers on the protoplasmic granules (Mullins, 1940).

The magnitude of the binding capacity of tissues was shown by Mazia and Mullins (1941). They immersed *Elodea* leaves in very dilute solutions of radioactive copper chloride and found that the cells were able to accumulate copper in excess of $3 \times 10^8$ of that present in the nutrient media. Mullins (1947) showed the rapidity of movement of substances within single cells of *Nitella* and *Chara* by dipping the ends of the cells into a solution containing radiophosphate and tracing movement by autoradiographic procedures.

Phosphorus uptake by yeast cells was shown to be almost entirely dependent upon carbohydrate metabolism (Mullins, 1942).

It was found possible to increase the radioactive phosphate exchange by yeast in the presence of glucose by the addition of trace amounts of riboflavin to the suspending medium. The increase observed ranged from 20 to 200 per cent over the phosphate exchange by controls using glucose alone. The presence of a phosphorus complexing factor at the cell's surface was shown by Nickerson and Mullins (1948).

The concept of base exchange, a problem directly in the realm of mineral nutrition, was also attacked with radioactive tracers. For many years it had been generally believed that exchanges of certain mineral nutrients between soil colloids and plant roots take place, but inability to distinguish atoms of one system from those of the other made it impossible to demonstrate it conclusively. Jenny and Overstreet (1939*a*, *b*) showed the extent of such an exchange and proposed a theory of contact exchange in which cations may transfer from clay to root and vice versa without entering the soil solution. These two authors with Ayers (1939) state further that " theories of chemical soil solution no longer suffice to explain fully the absorption of mineral elements by plants from soils. They must be supplemented by considerations of contact effects ". The rationale of the work by this group is presented by Overstreet and Jenny (1940). (See also Williams and Coleman, 1950).

Ions diffuse on the surface of colloidal particles and " jump " from one particle to another if the double layers interpenetrate. Roots may likewise participate (Jenny and Overstreet, 1939*a*). Overstreet, Broyer, Isaacs and Delwiche (1942) then showed that synthesized organic acids—and not carbonic acid—are the source of the hydrogen ions which replace the absorbed K on the clay. Carbonic acid was not involved in the end results of the accumulation reaction—but its participation in an intermediate reaction was not ruled out.

Vlamis and Pearson (1950) observed that the presence of $CO_2$ in a leaching solution of $H_2O$, nutrient solutions, etc. did not release either zirconium or niobium from the soil, but the presence of organic acids did. This is cited as additional evidence that excreted $CO_2$ does not function directly in mineral uptake by aiding exchange. Complete solution of this problem or formulation of a related one (see below on ion binding substances) would aid materially in clearing up a long-standing uncertainty.

By 1940 Hoagland was able to summarize his views on the de-

pendence between salt accumulation and metabolic activity in roots (Hoagland, 1940). Others of his group (Broyer and Overstreet, 1940) showed exchange to take place, both surface and deep seated, during periods of active (metabolic) salt accumulation, and in 1942 Hoagland and Broyer (1942) showed that cyanide, methylene blue and anaerobic conditions all suppressed salt accumulation, and that vacuolar accumulation did not occur anaerobically.

In some experiments with carrier free $Rb^{86}$ and $P^{32}$, Overstreet and Jacobson (1946) showed that non-metabolic rubidium uptake rapidly reached a limiting value, while $P^{32}$ approached a constant rate of uptake. Outgo was similar to uptake. This non-metabolic uptake was to a large degree confined to the first few millimeters of the root tip. That maximum absorption of $Sr^{85}$ and $I^{131}$ occurs within a few millimeters of the root apex was shown by Jacobson and Overstreet (1947). Dead roots showed the same pattern but accumulation was lower, and exchange for inert isotopes was much more rapid. The presence of ion-binding substances in the living protoplasm was indicated.

In an attempt to explain the rapidity with which one ion can be displaced from roots by a chemically related ion, and in addition to explain the apparent competition between the absorption of related ions, Jacobson et al. (1950) have postulated that an ion-binding substance may be present at the surface of roots. Competition for space between ions of a related character could then be explained. Unrelated ions may be bound by different ion-binding substances. This suggestion seems an inevitable one and has occurred a number of times (Hevesy, 1947*a;* Jacobson and Overstreet, 1947; Nickerson and Mullins, 1948; Hendricks, 1953). It has intriguing possibilities in helping to explain competition between similar ions for absorption " space ".

Broyer (1950) used radioactive Br in a study with excised barley roots, and confirmed again a rapid exchange reaction between the external medium and roots, but showed it to be relatively less important than migration by metabolic accumulation under favorable temperature conditions. At low temperatures exchange absorption predominated with high salt roots, but with low salt roots metabolic accumulation was more important. The movement of bromide across the root appeared to take place by either of two pathways. In low salt roots the path may be to vacuoles and

xylem. In high salt roots it is largely to xylem via the protoplasmic continuum. An exchange of phosphorus between root and nutrient solution amounting to 0.5 to 1.5% per day has been shown by Barbier and Husson (1952).

While excised roots have been of value in the study of certain aspects of the absorption problem, it does not follow that the information so derived can be projected to determine the site of absorption of the major fraction of the translocatable nutrients. As the study of the absorption mechanism matures, it is hoped that it will not be divorced from translocation, as this is the process which is responsible in a great measure for its continuation. Kramer and Wiebe (1952) have shown some departures from the view that the meristem is the principal site of mineral accumulation (specifically for $P^{32}$).

The absorption of fertilizer elements under field conditions can be studied to very good advantage by tracer methods. The nutrient environment can be treated as one system and the plant as another. In practice the tracer materials are mixed with the indigenous plant nutrients, the plants grown therein, and the plant parts then analyzed for both indigenous and tracer elements. This furnishes a basis for determining the efficiency of fertilizer uptake. This is a totally new approach to the fertilizer problem and offers the means for direct calculation of the percentage of applied element which makes up the final yield of plant material.

It is also likely that other methods of fertilizer application will become widespread. Particularly promising is the method of direct application of certain fertilizer elements to foliage. The feasibility of this method of application is based on the demonstration of the free and rapid movement of phosphorus (Biddulph, 1941; Biddulph and Markle, 1944) and of sulfur (Thomas, Hendricks and Hill, 1950) in plants. Other elements which are phloem mobile should be equally effective when applied in this manner. The feasibility of this method for the application of phosphorus has been recently demonstrated by Wittwer and Lundahl (1951), Silberstein and Wittwer (1951) and Eggert et al. (1952). The problem of a suitable method of application should present little difficulty.

The first work utilizing radioactive isotopes and dealing with strictly agricultural problems was completed by Henderson and

Jones (1941). They showed that $P^{32}$ applied to the soil surface as $Ca(H_2PO_4)_2$ penetrated as little as one and one-fourth inches into a clay soil and four inches into a silt loam when washed down with water equivalent to two and one-half inches of precipitation. Only 5% of the $K^{42}$ applied as KCl penetrated beyond one and five-eighths inches with the same addition of water. The authors conclude that " the radioactive technique appears to present a valuable method for studying the behavior of ions in soils ". This paper was followed immediately by Ballard and Dean's report (1941) that fixation of phosphorus in soils was much more tenacious than for sand cultures. Soil fixation approached that which was calculated by other means to be characteristic of phosphorus in the soils tested. Tomato plants were unable to utilize readily the so-called fixed phosphorus of soils, whereas Sudan grass was able to make some utilization of this fraction.

Comar and Neller (1947) developed radioactive phosphorus procedures for studies of soil fixation and uptake by plants of radiophosphorus distributed in soils; and Neller and Comar (1947) showed the fixation power of soils for phosphorus against a dilute acid extractant to be directly related to their clay contents. Fixation varied directly with the moisture equivalent but showed no correlation with pH values. MacKenzie and Dean (1948) have reported procedures and measurements for $P^{31}$ and $P^{32}$ in plant material. They included procedures for determining the specific activity of plant material containing radioactive phosphorus absorbed from $P^{32}$ treated soils. By comparison of the specific activity of the fertilizer preparation with that of the plants grown on soil receiving this fertilizer, the proportion of the phosphorus contained in the plant which was derived from the fertilizer was estimated. This allows a calculation of the fertilizer efficiency under different modes of application and by different crops.

In 1946 the Canadian group began a series of reports, the first of which (Spinks and Barber, 1946) pointed out that " it is possible to measure the phosphorus taken up from both fertilizer and soil and the utilization of the fertilizer phosphorus, something which could not possibly be done by ordinary chemical means ". In 1947 they (Spinks and Barber, 1947) showed a seasonal variation in uptake of fertilizer phosphorus by wheat with highest uptake when the roots were still in the zone of application and lowest

uptake during later stages when the roots had spread beyond the zone where the fertilizer was applied. In their experiments the main uptake of fertilizer phosphorus took place prior to the heading out stage. Approximately 22% of the fertilizer phosphorus was recovered by the plant. The next year's results (Spinks and Barber, 1948) led them to believe that most of the phosphorus taken up in the early stages of growth comes from the fertilizer. In 1948 (Spinks, Dion et al., 1948) they reported that the amount of fertilizer phosphorus taken up increased with its application, but the per cent uptake decreased. Also fertilizer application results in less uptake of indigenous phosphorus, ammonium phosphate being used in these experiments. Dion, Spinks and Mitcheli (1949) and Dion, Dehm and Spinks (1949) showed ammonium phosphate to be a particularly suitable carrier of phosphorus in the base-saturated soils of Saskatchewan. Mono-calcium and di-calcium salts gave decreasing effectiveness. A review of the above work has been published by Spinks and Dion (1949).

A study of phosphorus absorption by individual wheat and barley plants grown in test tubes has been reported by Naylor et al. (1951). An accounting of the deposition of the absorbed $P^{32}$ by each part is included.

Meanwhile the U.S.D.A. investigators had been perfecting a system for the use of $P^{32}$ in fertilizer studies, and their series of papers, published in 1947 (Hendricks and Dean, 1947; Nelson et al., 1947) furnishes a sound pattern for fertilizer studies with this element. A fertilizer is prepared with a known ratio of radioactive phosphorus to inert phosphorus. If a plant grown in soil to which this mixture has been added shows a ratio only one-fifth as great, then only one-fifth of the phosphate came from the fertilizer, the dilutant coming from the reservoir of indigenous phosphate in the soil. This "dilution" method is the basis of most fertilizer studies. It is ". . . so simple as to appear almost trivial, but it permits an entirely new approach to work with fertilizers". Some results employing this method show that plants derive a higher percentage of applied fertilizer phosphorus from soils of low phosphorus fertility than from soils with high phosphorus fertility (Dean et al., 1947). Potatoes, corn, cotton and tobacco vary greatly in absorption of fertilizer phosphorus on soils of comparable native phosphorus content. Also the period of greatest absorption, whether early in development or later, varied with the crop (Nel-

son et al., 1947). Exchange reactions between phosphate on the surface of soil minerals with phosphate in solution were also followed (McAuliffe et al., 1947). The results from their 1949 experiments, including information on $P^{32}$ absorption of potatoes, tobacco, corn, cotton, orchard grass, ladino clover, oats, alfalfa, sugar beet, wheat, barley and soybeans, are indicated as being tentative but tend to show that as roots grow through the fertilizer placement area the per cent of fertilizer phosphorus in plant parts decreases. Corn showed ten to 30% utilization of fertilizer phosphorus in early growth, falling to four to 15% by the roasting-ear stage (Stanford and Nelson, 1949*a*). In general the percentage of fertilizer phosphorus in the plant material was rather low, i.e., ladino clover 20% (Blaser and McAuliffe, 1949), potatoes four to 15% (Jacob et al., 1949) and cotton 5% on low phosphorus soils and 3% on high phosphorus soils (Nelson et al., 1949). Potatoes gave highest figures when compared under similar conditions to corn and soybeans (Krantz et al., 1949).

Banding or mixing the fertilizer with the soil (Woltz et al., 1949), especially at seed depth (Stanford and Nelson, 1949*a;* Nelson et al., 1949), was superior to broadcasting as a method of application. Drilled fertilizer was also superior to broadcasting (Blaser and McAuliffe, 1949). Deep placement was better than shallow (Olsen and Gardner, 1949).

Superphosphate appeared to give a higher percentage of fertilizer phosphate in plant parts than other forms including calcium metaphosphate, dicalcium phosphate and tricalcium phosphate (Blaser and McAuliffe, 1949; Hall et al., 1949; Stanford and Nelson, 1949*b;* Olsen and Gardner, 1949).

Another tendency was for the percentage of fertilizer phosphorus utilized by the plants to increase with the amount of fertilizer applied, especially in low phosphorus soils (Jacob et al., 1949; Woltz et al., 1949). That the application of fertilizer phosphorus increased yields and phosphorus content of plant parts was not always the case. Also included are studies on the utilization of farm manure labelled with radioactive phosphorus (McAuliffe and Peech, 1949; McAuliffe et al., 1949) and phosphorus uptake from green manure by rye grass (Fuller and Dean, 1949). (See also White, Fried and Ohlrogge, 1949.) These studies are the result of carefully planned experiments and, in general, show the possi-

bilities of applying tracer techniques to the general problem of fertilizer efficiency.

Fredriksson and Wiklander (1950) applied superphosphate containing $P^{32}$ to a sandy loam soil low in available phosphorus. They found that both yield and uptake of fertilizer phosphorus increased with the amount of phosphorus supplied. At high rates of application, broadcasting gave better yield than placement beneath the seed. Collier (1951) and Low (1951) have presented general reviews of the use of isotopes in agricultural research, and Noggle (1951) has prepared a general outline of possible tracer applications to soil studies. Utilizing a new technique for the study of the behavior of plant root systems, Hunter and Kelley (1946) showed that radiophosphorus was absorbed by a guayule plant root, at a depth of 48 inches, from soil which was below the permanent wilting percentage.

In studies of phosphorus uptake by grape vines under field conditions, Ulrich, Jacobson and Overstreet (1947) showed that phosphorus supplied at the surface was not so efficient as that applied at some depth in the soil. The calculations based on radioactive measurements indicated that less than one per cent of the phosphorus added to the soil was contained in the aerial parts. Soil analyses showed that the surface-supplied phosphorus had penetrated only 11 inches in 43 days, while the water had penetrated at least 20 inches.

Three papers by Japanese workers—Okuda, Kasai and Anma—show the distribution of absorbed $P^{32}$ in peanut and spinach (1951*a*), wheat (1951*b*) and sesame (1952). Early additions of marked phosphorus were found at harvest time to be accumulated in ears, leaves and stems, while later additions accumulated in roots.

### TRANSLOCATION

The geographic tracing of specific radioactive elements constituted a major part of the early work with tracers, and since translocation in plants is still poorly understood, much more of this type of study will be required. Marker or tracer materials may include essential or non-essential mineral nutrients, metabolites marked with $S^{35}$, $P^{32}$, or $C^{14}$, etc., growth regulators marked with $I^{131}$ or $C^{14}$, or water marked with tritium. In spite of the obvious con-

veniences of their use, the results obtained have not entirely measured up to reasonable expectations. The difficulty apparently stems from the lack of suitable methods of introducing them into the phloem. A solution of the problem would not only further translocation problems directly but would also contribute to furtherance of studies involving weed sprays, foliar application of fertilizers, hormone control of physiological processes, etc.

Perhaps the first major conclusive contribution to this field, and one which terminated a long period of uncertainty, was the clarification by Stout and Hoagland (1939) that the radioactive isotopes of potassium, sodium, phosphorus and bromine move upward through the xylem tissue. It was shown, however, that if wood and bark are in contact, the radioactive elements are rapidly transferred laterally from wood to bark. Gustafson and Darken (1937*a*, *b*) had previously made use of radiophosphorus to study the upward movement of this element in the stems of several plants. Their first conclusion was that radioactive phosphorus in the form of phosphate is transported upward through the phloem system. In a later experiment Gustafson (1939) reached the conclusion that not so much mineral matter was conducted through the phloem as he and Darken had previously supposed, but still maintained that some upward conduction through the phloem is possible under normal conditions.

Cyclotron produced radioactive sodium was used by Nisina and Nakayama (1938) to show the rapidity of movement of Na in roots and leaves of geranium, and in addition they cite experiments which are reported to show that salt rises in both phloem and xylem tissues.

Biddulph (1938, 1940) used radioactive phosphorus to show that upward movement in the stem of bean plants took place rapidly and followed the transpiration stream, with the greatest accumulation taking place in the uppermost leaves. The downward movement of this element in bean plants was then studied by means of a technique wherein the radioactive tracer was injected into a veinlet of a leaf in such a manner that back movement from the leaf took place through the phloem. The method is not without limitations, since it does open a vein and may under some conditions modify the normal hydrostatic forces, particularly in the xylem. The diurnal migration from the leaf was followed (Biddulph, 1941) and

it was shown that much the greatest movement occurred during the daylight hours. The possibility of a relatively rapid "circulation" of phosphorus within the plant was shown. By means of a stripping technique similar to that of Stout and Hoagland (1939) which was previously mentioned, Biddulph and Markle (1944) demonstrated the downward movement of phosphorus in the cotton plant to take place through the phloem. The rate of movement was well in excess of 21 cm. per hour, and marked phosphorus was also found in the phloem sections above the point where the phosphorus entered the stem, thereby showing that a simultaneous upward movement could also take place in the whole phloem tissue.

Colwell (1942) studied the movement of phosphorus from leaves of squash plants and reached the conclusion that the movement of the radioactive indicator was restricted to the phloem and that the movement was correlated with food movement in the plant. Some additional information bearing on the movement of phosphorus in stems was obtained by Moore (1949) by the use of corn plants whose roots were divided in such a manner that each half of the root system was maintained in a different nutrient solution. Then by adding radioactive phosphorus to one solution he found that the marked phosphorus readily ascended the stem and after six hours some of it had descended into the opposite group of roots. Some labelled phosphorus could be detected in the solution bathing these roots after approximately 96 hours.

There is considerable advantage to be gained by the simultaneous use of two tracers in the same plant section. Rabideau and Burr (1945) made use of the stable carbon isotope $C^{13}$ and radioactive phosphorus to study the simultaneous movement of carbohydrates and phosphorus. They found a rapid transport of labelled photosynthate both upward and downward in the stem to the metabolically active regions of root tip and stem tip. In its upward movement radioactive phosphorus passed through killed areas of bean stems, while compounds containing $C^{13}$ did not. An additional contribution to this problem was made by Chen (1951). He obtained with willow cuttings substantially the same results as were obtained by Stout and Hoagland (1939). In addition, by supplying $C^{14}O_2$ to leaves of geranium plants at one level on the stem and $P^{32}$ to leaves at a different level, he detected simultaneous but directionally opposite movement of $C^{14}$-labelled photosynthate and

$P^{32}$ in the phloem. Since the stem had been previously stripped, the movement was shown to have taken place in the phloem tissue.

The factors which influence the release of mineral nutrients from roots into xylem tissue are not well understood, but some attempts have been made to determine the conditions which most favor this release. Broyer and Hoagland (1943) showed that large differences in transpiration had little influence on movement into the shoot, but they report that, under some conditions, reducing transpiration to the greatest possible extent may, for short periods at least, prevent movement of salts to the upward part of the shoot. They concluded that the role of transpiration in upward movement is not excluded despite the importance of metabolic factors. Broyer (1950) showed that in low salt plants, salts may migrate to vacuoles or to the xylem areas. In high salt plants migration along the symplast to the xylem takes place.

Hanson and Biddulph (1953) found a diurnal variation in the amount of rubidium and phosphate which was translocated to the shoot in bean plants. The maximum occurred near midday and the minimum near midnight. Superimposed on the diurnal pattern are effects due to the metabolic status of the roots. Those roots which absorb the most ion, by reason of adequate supply of metabolite or a low salt–high sugar nutritional status, will also translocate a greater percentage of the ion to the shoot. Xylary translocation of radiophosphorus in pine has been correlated with anatomical structure to explain an observed difference in the rate of movement within stems and roots (Moreland, 1950).

As previously mentioned, Hevesy, Linderstrom-Lang and Olsen (1936, 1937) employed an isotope dilution technique which demonstrated the rapid interchange of phosphorus atoms between parts of both corn and sunflower plants. A similar experiment using $N^{15}$ showed rapid interchange of $N^{15}$ into proteins as well as rapid geographic interchange between leaves (Hevesy et al., 1940). Using wheat seedlings with parted roots, one part being placed in an inactive solution and the other in a radioactive phosphorus-containing solution, Hevesy (1947*b*) showed a migration through the plant from the active to the inactive solution.

That translocation studies have progressed to the stage where it is possible to discuss circulatory disorders in plants is the object of a report by Biddulph (1951). Particular attention is given to

the effects of phosphorus and other factors on the mobility of radio-iron in bean plants. Rediske and Biddulph (1953) have pointed out that iron is almost completely immobilized in bean plants grown at medium to high phosphorus levels, and a pH of 7.0 or above. Iron is readily phloem mobile only if the plants are low in phosphorus and iron, and are growing in an acid medium near pH 4.0. Some additional information on the mobility of phosphorus, sulfur, calcium and zinc is reported in a later publication (Biddulph, 1953). Phosphorus and sulfur were shown to be particularly mobile in the phloem, iron and zinc conditionally mobile and calcium practically immobile.

## METABOLISM

CALCIUM. The radioactive isotopes of calcium and their suitability as indicators in biological systems were described by Walke, Thompson and Holt (1940). They describe the isolation of the various calcium isotopes, including $Ca^{45}$, from bombarded materials. Since that time little use has been made of this element in plant studies. Bledsoe, Comar and Harris (1949) used $Ca^{45}$ to study the uptake of calcium by peanut fruit. They showed a very limited movement of root-absorbed calcium into the developing fruit, but when tracer calcium was placed in the environment of the young pegs, the developing fruits readily absorbed it. The failure of root-absorbed calcium to move into the fruits indicated a lack of mobility of calcium in the phloem, which is in keeping with the general opinion that calcium is not readily phloem mobile. The developing peanut fruit is then dependent upon direct absorption of calcium and does not utilize that which is absorbed by the root. Biddulph (1953) has also demonstrated that calcium is almost completely immobile in the phloem of the bean plant.

Procedures for the use of $Ca^{45}$ in plant and animal studies have been presented by Comar et al. (1951). Calcium has not been extensively used as a tracer in plant studies. It offers a number of advantages which would warrant much more extensive use: it has a long half-life, i.e., 180 days, and can be obtained in sufficiently high specific activity for most biological studies.

COPPER. Mazia and Mullins (1941) immersed *Elodea* leaves in very dilute solutions of radioactive copper chloride ($Cu^{86}$) and found that the cells were able to accumulate copper in excess of

$3 \times 10^8$ of that present in the nutrient media. They conclude that the effects of copper are due to the power of protoplasm to bind these ions against a concentration gradient rendering them available for the physiological processes in which they participate.

IODINE. Many uses have been made of radioactive iodine in animal studies, and it has been employed in connection with other radioisotopes in several plant studies; but the only attempt to study the metabolism of radioactive iodine in plants was made by Kelly and Baily (1951) for brown algae. By the use of the tracer it was shown that a steady state of iodine content is reached, after which further increase does not occur. There is a continual exchange between the iodine within the plant segment and the iodine in the surrounding medium.

IRON. Manganese interference in the absorption and translocation of radioactive iron ($Fe^{59}$) in pineapple has been reported by Sideris (1950). Most iron which was removed from the nutrient solution was deposited in the roots, especially in the cultures containing manganese. The amounts of iron translocated from the root to the leaves was correspondingly lower in the plants grown in the presence of manganese. Considerable amounts of the translocated iron remained in the proteinaceous matter of the cell.

Rediske and Bidulph (1953) studied the absorption and translocation of radioactive iron ($Fe^{55}$) in bean plants. The factors which influenced the distribution of iron in a plant were discussed, and it was shown that iron could be readily immobilized within the tissue or that it could remain free for translocation. The factors which tended toward immobility were high phosphorus content of the tissue and growth in a nutrient media at pH 7 or above. Iron remained freely mobile in those plants wherein the iron and phosphorus content of the tissue was low and their roots were immersed in an acid nutrient medium of approximately pH 4. Autoradiographs of chlorotic and green leaves show the different distribution patterns of iron in these instances.

MANGANESE. Millikan (1951) has presented a rather comprehensive picture of the behavior of radioactive manganese ($Mn^{54}$) in flax, peas, cabbage and tomato plants. A number of cases of irregular distribution of manganese in senescent tissues are described as well as the distribution in normal healthy tissue. Suc-

cessive autoradiographs of the same pea plant showed that manganese moves from the interveinal tissue into the veins as the plant dries out. This indicates that some caution is required in preparing plant parts for autoradiographs if the drying procedure is followed.

MOLYBDENUM. Stout and Meagher (1948) present a complete accounting of the absorption and subsequent distribution of a single microgram of molybdenum in a growing plant. They show that it is readily absorbed and translocated, as are other microelements such as manganese and zinc. They present autoradiographs showing the comparative distribution of $K^{43}$, $Mo^{93}$ and $Mo^{99}$, and conclude that molybdenum shows a different type of distribution within plant tissue than do the other mineral nutrients, potassium, phosphorus, manganese and zinc. Phosphorus was reported to have a distinct influence on the absorption of molybdenum.

PHOSPHORUS. The first use of radioactive phosphorus in plant studies was reported by Hevesy, Linderstrom-Lange and Olsen (1936, 1937). They employed an isotope dilution technique in order to show the exchange of phosphorus atoms between older and younger leaves of corn and sunflower, respectively. This marked the beginnings of the use of this element in the tracing of metabolic paths in plants. The early work with this element was largely from cyclotron-produced phosphorus 32. The cyclotron-produced radiophosphorus persisted as the sole practical source of this element until after the diversion of some of the pile facilities for the manufacture of this and other radioactive elements at the termination of the recent war. The quantities which were available were limited, as compared to present supply, but served admirably for certain types of studies involving migration and deposition.

Brewer and Bramley (1940) and Brewer (1941) used radioactive phosphorus and sodium to show the rapidity of appearance of these elements in leaves after their administration to the roots. They also showed some back diffusion of sodium from the plant to the nutrient medium. Only a very slow back diffusion of phosphorus occurred. Arnon, Stout and Sipos (1940) made a more extensive study of the distribution of phosphorus in tomato. Phosphorus was shown to move from the solution to the tops of six-foot tomato plants within 40 minutes. The greatest capacity for phos-

phorus uptake was shown by young fruits, although the element moved readily to all parts of the plant.

In a study of the effect of the form of available nitrogen on the absorption and distribution of phosphorus by the tomato plant, Breon et al. (1944) showed that plants furnished urea absorbed $P^{32}$ at a much more rapid rate than those furnished with nitrate. Also those plants lowest in phosphorus absorbed $P^{32}$ the fastest. The distribution of the absorbed $P^{32}$ in the ether soluble, alcohol soluble, water-acid soluble, and the insoluble fraction is given. Phosphorus compounds synthesized by the plants were in about the same proportion for the phosphorus deficient and normal phosphorus plants, but the rate of synthesis was faster in phosphorus deficient plants. Biddulph and Brown (1945) followed the movement of phosphorus into developing cotton flowers and attempted to correlate the movement of phosphorus into the developing flowers and fruits with the morphological events of synapse and fertilization. It was shown that the highest concentration of phosphorus was in young primordia and decreased progressively thereafter. Small gains were made with each morphological event, but on the basis of per cent the phosphorus continued to decrease with maturation of fruits. Withner (1949) presents a dynamic picture of phosphorus movement into various parts of corn plants during the course of their development.

Some of the factors which influence the uptake of phosphorus by bean plants were studied by Biddulph and Woodbridge (1952). The distribution of phosphorus in this plant as influenced by pH and phosphorus concentration was reported. The presence of iron in the nutrient media had some influence over the amount of phosphorus available for metabolic use when the supply of this element was extremely limited. The distribution of phosphorus in soybeans was shown by Klechkovskii et al. (1947). Their study compared the distribution which was obtained by plants having adequate phosphorus nutrition with plants on a sharply curtailed supply.

An interesting application of radiophosphorus has been made by Sisakyan and Voronkova (1950) and by Klechkovskii, Stoletor and Evdokimova (1951) in an attempt to study the compatibility between the stock and scion of grafted plants. This idea might easily be extended to a study of the mechanism of dwarfing of certain stocks; of resistance to chlorosis, virus diseases, etc.

Studies of phosphorus absorption and distribution in inbred and hybrid maize have been reported by Rabideau et al. (1948, 1950). It is interesting to note that a tracer can be used to show differences in phosphorus metabolism by these genetically different plants. Tsao, Rabideau and Whaley (1950*a*) have also studied the phosphorus uptake of *Andropogon* species at various stages of development, and the effect of phosphorus nutrition on nitrogen absorption of four species of grass (1950*b*).

The absorption of radioactive phosphorus by mycorrhizal roots of pine was studied by Kramer and Wilbur (1949). They showed that mycorrhizal portions of pine roots accumulated much larger quantities of phosphorus than non-mycorrhizal portions. Kramer (1951) later studied the effects of respiration inhibitors on the accumulation of $P^{32}$ by mycorrhizal and non-mycorrhizal roots of pine and reached the conclusion that, in general, pine roots seem to react to respiration inhibitors in about the same manner as barley and tomato roots. The non-mycorrhizal roots appeared more sensitive to azid and fluoride inhibitors than mycorrhizal roots. Tsao and Whaley (1950) report that plant gall tissue accumulates more phosphorus than healthy adjacent tissue and suggest that metabolic differences may be responsible for the accumulation.

A very interesting application of phosphorus-containing fertilizers as foliar amendments has been made by Silberstein and Wittwer (1951), who have shown that foliar applied phosphorus was utilized much more effectively than phosphorus applied broadcast to the soil, even though the latter treatment gave highest total yields. A concentration of 25 to 50 millimoles of phosphorus per liter was found most desirable for the greatest movement of the compounds which were tried. Generally, *o*-phosphoric acid was the most effective of all chemicals tried. The foliar phosphorus was traced to all parts of the plant, including root tips, and to the areas of high metabolic activity. Apparently within 48 hours after treating, five to six per cent of the total phosphorus in developing tomato fruit may be derived from a single foliar application of *o*-phosphoric acid. Eggert, Kardos and Smith (1952) applied tracer phosphorus to apple trees, both as foliar sprays and as soil amendments. A single foliar application resulted in uptake amounting to two to three per cent of the total $P_2O_5$ of the trees. There was continual absorption for 30 days after application, even though

no subsequent rain or water spray was used to moisten the leaves. About half of the leaf-absorbed phosphorus was translocated to the root; the cores, seeds and subepidermal tissues of the fruit were the highest of the aerial tissues. No uptake of soil-applied tracer phosphorus could be detected under any of the regimes tried. The tracer phosphorus was more efficiently absorbed from diammonium phosphate than from several other phosphorus-containing compounds. The mobility of phosphorus within bean and cotton plants was shown earlier (Biddulph, 1941; Biddulph and Markle, 1944) and this type of application is a natural development for elements which are readily phloem mobile (Biddulph, 1953).

SULFUR. The first use of radioactive sulfur in plant studies was by Thomas et al. (1944). These authors used sulfur in the form of sulfur dioxide in order to show the uptake of it through the aerial portions of the plant. They found a high initial absorption of sulfur dioxide by the leaves, followed by a lowering of the concentration as the sulfur was distributed throughout the plant. Translocation was assumed to be in the form of sulfate. Root absorption studies, using a single application of radioactive sulfur as sodium sulfate, showed that the concentration of the leaves built up to a maximum in eight to ten days. This was followed by a lowering of the concentration as sulfur was redistributed. They found that top concentrations were higher than root concentrations for both wheat and barley. Distribution of sulfur was also studied in corn; the highest concentration was in the leaves with the least in the stalks and husks. Because of the stage of maturity, distribution in the developing ear was not studied. The radioactive sulfur was traced into the various fractions—sulfate, acid soluble, acid insoluble and labile sulfur.

In connection with this work, Harrison, Thomas and Hill (1944) used an autoradiographic procedure which was able to show the distribution of sulfur in the developing tissues of wheat. Rather uniform distribution of sulfur in wheat leaves was reported, but the highest concentrations were found in the kernel which was sampled at the dough stage. There was a particularly marked concentration of sulfur in the embryo and in the periphery of the endosperm, especially the aleurone layer. The distribution through the tissues appeared to be similar, whether the sulfur entered as sulfur dioxide or as sodium sulfate.

The sulfur metabolism of alfalfa was also studied (Thomas et al., 1950). Their experiment showed that in sulfur-deficient plants a maximum concentration in the leaves was reached in 16 days. Sulfate was gradually converted into the organic form. When the plants were cut back and the new shoots exposed to sulfur dioxide there was a very rapid uptake of radiosulfur with a corresponding rapid conversion into the organic form. The sulfate in the roots was not changed into organic forms unless top growth was present.

A translocation experiment was conducted by dividing the shoots and dipping the tops of one group into a solution of radiosulfate. The absorbed sulfate was translocated down the stem where it was changed steadily into the organic form. Some sulfur passed down through the crowns or roots and reappeared in the leaves of the undipped stems where it was also changed into the organic form.

Fried (1948) studied the absorption of sulfur dioxide by the aerial parts of alfalfa plants. He sealed the soil surface in order to isolate the roots and tops from each other in an attempt to exclude the possibility of root absorption of sulfur dioxide. He confirmed the finding that alfalfa plants can take in sulfur dioxide through the leaves and convert it into organic sulfur compounds. Autoradiographs were used to show the distribution of sulfur in the plant parts.

The amount of absorbed sulfur necessary for destroying rust and mildew infections without injury to the host was determined by Yarwood and Jacobson (1950), using sulfur 35. One to three $\mu$gm. of sulfur per square centimeter of leaf area was required while the leaf could stand from three to six $\mu$gm. with only slight injury. They concluded that selective absorption by diseased tissue (host and pathogen) may explain the chemotherapeutic effect.

Sulfur has been used frequently as a fumigant for various fruits, and it has been shown by use of $S^{35}$ that sulfur vapor penetrates into citrus fruits and is incorporated into various sulfur-containing compounds (Turrell and Chervenak, 1949). In a rather exhaustive study Turrell (1950) has shown that various environmental factors may influence the relative distribution of the radioactive sulfur used as a fumigant in the sulfur compounds of the fruit.

SODIUM. Data on radioactive sodium ($Na^{22}$) uptake per cm.$^2$ of leaf area as a function of the sodium concentration in the nutrient medium is given for corn by Brewer (1941), and the inter-

relationship of sodium and potassium in plant nutrient studies was shown by Leonard and Toth (1950) by the use of $Na^{22}$. The radioactive sodium tended to concentrate in the conducting tissues of the plants. The salient features of the $Na^{22}$ distribution in several species of plants were described. Both activity measurements and autoradiographs were used to portray the distribution.

ZINC. A study of the metabolism of $Zn^{65}$ in peas by Bergh (1951) shows that the root had the highest zinc content and the older leaf blades the lowest. Seventy-seven per cent of the zinc associated with the root remained insoluble, whereas in the aerial portions—shell, leaf blades and stems—65 to 85% of the zinc was soluble in cold water. The insoluble residue appeared to be fixed in the proteins. See also Stout et al. (1947) for a study of the zinc metabolism of tomato.

## RADIATION INJURY

In this discussion no attempt is made to survey properly or review the effects of radiation on plants. However, insofar as the radiation emitted by "tracers" may influence the physiological process studied, it seems desirable to include that literature which establishes or attempts to establish a safe level of radiation for tracer studies. It is important not to employ a tracer yielding sufficiently intense radiation to disrupt or alter the process under consideration.

Radiation from the natural radioactive materials has long captivated the imagination, and it appears to have been difficult, at least for the uninitiated, to refrain from assigning magical powers to it—either for good or for evil. In the early literature, Gager (1907) became interested in atomic disintegrations (radioactive) vs. molecular disintegrations (destructive metabolism) and the crude analogies between them. While it was claimed that radioactivity is in no sense comparable to metabolism, it was considered desirable to establish the fact that radioactive substances are incapable of independently elevating inorganic compounds to the state of those found in living organisms. There are a number of reports extending to relatively recent times (Leggieri, 1940), claiming that physical effects from radioactive waters were apparent. Brief immersion of vetch beans and corn in radioactive water was claimed to have a stimulative effect, but prolonged immersion was unfavorable.

The list of physiological processes which were influenced was rather long. It has been very difficult to confirm or deny that radiation from relatively weak sources has stimulative properties. The difficulties encountered are not only inaccurate measurement of the radiation but also quantitative appraisal of plant behavior.

The influence of naturally occurring radioactive elements on growth and yield of plants has been reported by Drobkov (1937, 1939, 1940). Increases in yield of peas up to 182% in the first and as high as 204% in a second report are claimed. Even nitrogen fixation by legumes is reported (1945) to have been influenced. French (1948), as the result of experiments on five vegetables, concludes that the use of radioactive materials in conjunction with commercial fertilizers tends to increase the mean yield of many crops.

Perhaps the most comprehensive survey of the effect of radioactive materials on yields of plants has been made by Alexander (1950) who attempted on a sound and systematic basis to appraise the effect of radioactive stimulants on the yield of plants in the field. Alpha rays from actinium in "alphatron" together with radium and uranyl nitrate were the three sources of radiation. After careful experimentation, the general conclusion to be drawn from the data was that no effect of the radioactive materials was found either beneficial or harmful. There were a few cases where differences reached the five per cent level of significance, but they were no more than would be expected in sampling from normal distribution.

Until further carefully conceived experiments are designed which yield evidence to the contrary, the writer feels it justifiable to conclude that stimulatory effects resulting from radiation have not been established.

Simultaneously with the use of radioactive tracers it became desirable to attempt an establishment of the level at which radiation damage first occurred. Since radiation effects on living tissue are extremely complex, a satisfactory quantitative test for injury was not immediately apparent. It became necessary as each physiological process was studied to determine the effects of radioactivity from the tracers on that particular process. As a result, there is accumulating in the literature considerable quantitative data on the effect of ionizing radiation on living tissue.

Brooks (1939) showed that there is no observable effect on oxygen consumption, as indicated by the Warburg method when *Nitella* was exposed to the radioactivity of sodium between the limits of 2.2–12 mc./l. Mullins (1939*a*) showed that radiation from radioactive sodium decreased the amount of penetration of sodium ions into *Nitella* cells when the concentration of the radioactive ion exceeded one mc./l. The decrease was principally of "induced accumulation" due to a decrease in the number of exchange positions of the protoplasm. Radioactive $K^{42}$ could also be shown to produce the same effect (Mullins, 1939*a*). Muir (1942) showed that beta radiation from radioactive phosphorus increased the viscosity of the protoplasm of *Spirogyra* when the strength of the radioactive solution was four to 17 mc./l. but had no effect when the radioactive strength was 2.1 mc./l. Some changes noted in total phosphorus may have resulted from effects upon permeability of beta radiation from the radioactive isotopes.

Weintraub (1944), in a review of radiation and plant respiration, concludes that under some conditions an increase in the rate of "apparent" respiration, as measured by gaseous exchange, may be induced by radiation of various species of plants and types of tissue. He was unable to decide whether the observed stimulation was directly related to "true" respiration. He concludes with the statement that, "Despite the long continued interest in this problem, the results available are almost entirely of a descriptive nature and in no single case has there been presented, as yet, a satisfactory illucidation of the mechanisms involved".

Spinks et al. (1948) used $P^{32}$ and $Sr^{90}$ as radioactive sources and observed a lethal limit of absorbed radioactive isotope between 0.0065 Rd per seed and 0.065 Rd per seed for wheat, barley and sunflower in the seedling state. Sunflower proved somewhat more resistant than wheat and barley. Dion et al. (1949) used radioactive fertilizer phosphorus at 26 mc. and 260 mc. per gram of phosphorus and concluded that there were neither harmful nor beneficial effects observable at this rate of application. Russell and Martin (1949) used $P^{32}$ at activities between 0.5 and 50 $\mu$c./l. As a result of these investigations they concluded that radiation damage may be a much more serious hazard than previous literature had led them to believe. They found significant radiation effects when the level of $P^{32}$ is ten $\mu$c./l. These authors worked with rapidly

growing young plants where phosphorus accumulation was high. Blume et al. (1950) used barley grown in nutrient culture exposed to $P^{32}$. They concluded that damage to tops was more severe than damage to roots and that injury was due to the radiation from $P^{32}$ accumulated in the plant; the $P^{32}$ remaining in solution had relatively little effect. Damage to cells was manifested in the zones of active cell division, and the lowest level at which damage was discernible corresponded to 5.6 mc./gm. of $P_2O_5$.

Bould, Nicholas and Thomas (1951) concluded that radiation effects on the growth and physiology of barley and tomato do occur at low radioactive dosages, lower than those accepted as "safe" by some investigators. They state that the most striking feature of their experiments was the smallness of the radiation effect despite the fact that they worked at concentrations of $P^{32}$ in excess of those which would normally be used in tracer work. They were not concerned with morphological or cytological abnormalities which may have occurred in tissue, but considered only the criteria which would be used in plant nutrition studies in soils.

Some effects of high concentrations of $P^{32}$ on the growth of potato have been reported by Stanton and Sinclair (1951). Dosages of the order of 3,000 r received by the growing points in the first fortnight were responsible for rather striking abnormalities. The abnormalities included increased fleshiness of leaves, loss of secondary leaflets, increased branching and the appearance of what is termed "blind" plants, in which the main shoot terminates in three leaves surrounding an aborted growing point.

Because of the interest in the possible spread of fission products as a result of atomic weapons and disposal problems associated with the manufacture of plutonium, it becomes desirable to investigate the effects of fission products on plants. Jacobson and Overstreet (1948) have made a study of uptake of fission products and plutonium adsorbed in soil colloids. They found that barley and pea plants were able to take up fission elements Y, Ce, Zr, Cb, Sr and the three valence states of Pu, even when these elements were present in trace amounts on the surfaces of clay or soil particles. The greatest fixation is in or on the roots, and with the exception of Sr, translocation occurs only to a limited extent. The translocation of Sr is relatively large, i.e., 0.1 μc./gm. of soil acting over three months supplied sufficient strontium to the tops to show

" radiation injury ". Within the soil, activity levels of 0.1 μc./gm. of soil were sufficient to cause very pronounced injury over a three months' period.

Mackie et al. (1952) have shown that " plants grown in solutions of comparatively low specific activity exhibited clear evidence of damage to shoot meristems, but no apparent injury to root tips. Measurements . . . gave no indication of a threshold value for radiation effect upon meristematic cells ". " By extrapolation . . . one might expect that some injury to the plant would occur from the application of any amount of ionizing radiation, regardless of how small. It does not follow that this injury, when slight, would be reflected in more permanent symptoms of abnormality ".

There is a very lively interest in the biological effects of radiation at the present time. This subject is far too extensive to be reviewed here. My desire is merely to point up the need for careful consideration in choosing levels of radioactivity which will not invalidate the results expected from a particular experimental design.

## BIBLIOGRAPHY


ALEXANDER, LYLE T. 1950. Radioactive materials as plant stimulants—field results. Agron. Jour. **42**: 252–255.

ARNON, D. I., P. R. STOUT, and F. SIPOS. 1940. Radioactive phosphorus as an indicator of phosphorus absorption of tomato fruits at various stages of development. Am. Jour. Bot. **27**: 791–798.

BALE, W. F., F. L. HAVEN, and M. L. LEFEVRE. 1939. A method for measurement of radioactivities of solutions by means of the Geiger-Müller counter. Rev. Sci. Inst. **10**: 193.

BALLARD, S. S., and L. A. DEAN. 1941. Soil studies with radioactive phosphorus: Significance of biological measurements of the retention of applied phosphorus by soils. Soil Sci. **52**: 173–183.

BARBIER, G., and C. HUSSON. 1952. Use of phosphorus$^{32}$ in the study of the phosphate nutrition of plants. Acad. Sci. Compt. Rend. **234**: 126–128.

BARNES, T. C., and T. L. JAHN. 1934. Properties of water of biological interest. Quart. Rev. Biol. **9**: 292–341.

BECQUEREL, H. 1896. Acad. Sci. Compt. Rend. **122**: 420, 501, 559, 689, 762, 1086.

BELANGER, L. F., and C. P. LEBLOND. 1946. A method of locating radioactive elements in tissues by covering histological sections with a photographic emulsion. Endocrin. **39**: 8–13.

BERGH, HELGE. 1950. Metabolism of zinc with radiozinc$^{65}$; some investigations with radiozinc given to *Pisum* s. *saccharatum*. Kgl. Norske Videnskab. Selskab. Forh. **23**: 123–126. [Chem. Abs. **46**: 3121–b. 1952.]

BIDDULPH, O. 1939. Movement of radiophosphorus in bean seedlings. Science **89**: 393–394.

———. 1940. Absorption and movement of radiophosphorus in bean seedlings. Plant Physiol. **15**: 131–136.

———. 1941. Diurnal migration of injected radiophosphorus from bean leaves. Am. Jour. Bot. **28**: 348–352.

———. 1951. The translocation of minerals in plants. *In:* Truog, E. (ed.), Mineral nutrition of plants.

———. 1953. Translocation of radioactive mineral nutrients in plants, *Use of Isotopes in Plant and Animal Research.* Kansas Agr. Exp. Sta. Report No. 4, pp. 48–58. Kans. St. Col., Manhattan, Kans. (U.S.A.E.C. TID 5098).

——— and D. H. BROWN. 1945. Growth and phosphorus accumulation in cotton flowers as affected by meiosis and fertilization. Am. Jour. Bot. **32:** 182–187.

——— and J. MARKLE. 1944. Translocation of radiophosphorus in the phloem of the cotton plant. Am. Jour. Bot. **31:** 65–70.

——— and C. G. WOODBRIDGE. 1952. The uptake of phosphorus by bean plants with particular reference to the effects of iron. Plant Physiol. **27:** 431–444.

BLASER, R. E., and C. MCAULIFFE. 1949. Utilization of phosphorus from various fertilizer materials: I. Orchard grass and ladino clover in New York. Soil Sci. **68:** 145–150.

BLEDSOE, R. W., C. L. COMAR, and H. C. HARRIS. 1949. Absorption of radioactive calcium by the peanut fruit. Science **109:** 329–330.

BLUME, JAMES M., C. E. HAGEN, and RUTH W. MACKIE. 1950. Radiation injury to plants grown in nutrient solutions containing $P^{32}$. Soil Sci. **70:** 415–426.

BORKOWSKI, C. J. 1949. Instruments for measuring radioactivity. Anal. Chem. **21:** 348–352.

BOULD, C., D. J. D. NICHOLAS, and W. D. E. THOMAS. 1951. Radiation effects in plant nutrition experiments with phosphorus$^{32}$. Nature **167:** 140–142.

BOURNE, G. H. 1952. Autoradiography. Biol. Revs. Cambridge Phil. Soc. **27:** 108–131.

BOYD, G. A., and F. A. BOARD. 1949. A preliminary report on histochemography. Science **110:** 586–588.

——— and H. LEVI. 1950. Carbon$^{14}$ beta track autoradiography. Science **111:** 58–59.

BRANSON, H. 1947. The use of isotopes to determine the rate of a biological reaction. Science **106:** 404.

BREON, W. S., W. S. GILLAM, and D. J. TENDAM. 1944. Influence of phosphorus supply and the form of available nitrogen on the absorption and distribution of phosphorus by the tomato plant. Plant Physiol. **19:** 495–506.

BREWER, A. K. 1941. Isotopes in the study of plant growth. Jour. Chem. Ed. **18:** 217–221.

——— and A. BRAMLEY. 1940. A radioactive isotope study of the absorption of phosphorus and sodium by corn seedlings. Science **91:** 269–270.

BROOKS, M. 1939. Effects of certain radioactive elements on the metabolism of cells. Proc. Soc. Exp. Biol. & Med. **42:** 558–559.

BROOKS, S. C. 1937. Selective accumulation with reference to ion exchange by the protoplasm. Trans. Faraday Soc. **33:** 1002–1006.

———. 1938a. The chemical nature of the plasma membrane as revealed by permeability. Am. Nat. **72:** 124–140.

———. 1938b. Penetration of radioactive ions, their accumulation by protoplasm of living cells (*Nitella coronata*). Proc. Soc. Exp. Biol. & Med. **38:** 856–858.

———. 1938c. The penetration of radioactive potassium chloride into living cells. Jour. Cell. & Comp. Physiol. **11:** 247–252.

———. 1939. Ion exchanges in accumulation and loss of certain ions by the living protoplasm of *Nitella.* Jour. Cell. & Comp. Physiol. **14:** 383–401.

————. 1940. The intake of radioactive isotopes by living cells. Cold Spring Harbor Symp. Quant. Biol. **8**: 171–180.

BROYER, T. C. 1950. Further observations on the absorption and translocation of inorganic solutes using radioactive isotopes with plants. Plant Physiol. **25**: 367–376.

———— and D. R. HOAGLAND. 1943. Metabolic activities of roots and their bearing on the relation of upward movement of salts and water in plants. Am. Jour. Bot. **30**: 261–273.

———— and R. OVERSTREET. 1940. Cation exchange in plant roots in relation to metabolic factors. Am. Jour. Bot. **27**: 425–430.

BURCH, G., P. REASER, T RAY, and S. THREEFOOT. 1950. A method of preparing biologic fluids for counting of radioelements. Jour. Lab. Clin. Med. **35**: 626–630.

CHEN, S. L. 1951. Simultaneous movement of phosphorus$^{32}$ and carbon$^{14}$ in opposite directions in phloem tissue. Am. Jour. Bot. **38**: 203–211.

COHN, W. E. 1948. Radioactive contaminants in tracers. Anal. Chem. **20**: 498–503.

COLLIER, P. A. 1951. The use of isotopes in agricultural research. I. Chem. & Ind. **51**: 1122–1124.

COLWELL, R. N. 1942. The use of radioactive phosphorus in translocation studies. Am. Jour. Bot. **29**: 798–807.

COMAR, C. L., S. L. HANSARD, S. L. HOOD, M. P. PLUMLEE, and B. F. BARRENTINE. 1951. Use of calcium-45 in biological studies. Nucleonics **8**: 19–31.

———— and J. R. NELLER. 1947. Radioactive phosphorus procedures as applied to soil and plant research. Plant Physiol. **22**: 174–180.

CURIE, (MME.) MARIE SKLODOWSKA. 1898. Rayons émis par les composés de l'uranium et du thorium. Acad. Sci. Compt. Rend. **126**: 1101–1103.

DEAN, L. A., W. L. NELSON, A. J. MACKENZIE, W. H. ARMIGER, and W. L. HILL. 1947. Application of radioactive tracer technique to studies of phosphatic fertilizer utilization by crops. I. Greenhouse experiments. Proc. Soil Sci. Soc. Am. **12**: 107-112.

DION, H. G., C. F. BEDFORD, R. J. ST. ARNAUD, and J. W. T. SPINKS. 1949. Plant injury from $P^{32}$. Nature **163**: 906–907.

————, J. E. DEHM, and J. W. T. SPINKS. 1949. Study of fertilizer uptake with radioactive phosphorus. IV. The availability of phosphate carriers in calcareous soils. Sci. Agr. **29**: 512–526.

————, J. W. T. SPINKS, and J. MITCHELL. 1949. Experiments with radiophosphorus on the uptake of phosphorus by wheat. Sci. Agr. **29**: 167–172.

DROBKOV, A. A. 1937. [The influence of the radioactive elements, uranium, radium, thorium and actinium on the yield of plants]. Compt. Rend. Acad. Sci. U.R.S.S. **17**: 229–232. [Chem. Abs. **32**: 2569–9. 1938].

————. 1939. [The effect of uranium, radium and actinium on the yield of peas]. [Trav. Lab. Biogeochim. Acad. Sci. U.R.S.S.] **5**: 201–204. [Chem. Abs. **34**: 4113– . 1940].

————. 1940. [The influence of radioactive elements on the yields of plants]. [Bull. Acad. Sci. U.R.S.S., Ser. Biol.] 783–801. [Chem. Abs. **37**: 2776–3. 1943].

————. 1945. [The effect of radioactive elements upon development of root-nodule bacteria and upon the assimilation by them of the molecular nitrogen of the atmosphere]. [Compt. Rend. Acad. Sci. U.R.S.S.] **49**: 224–226. [Chem. Abs. **40**: 4772–4. 1946].

DUBRIDGE, L. A. 1938. Some aspects of nuclear physics of possible interest in biological work. Jour. App. Phys. **9**: 178–186.

DUGGAR, W. M., JR., and D. E. MORELAND. 1953. An autoradiographic technique for detailed studies with plant tissue. Plant Physiol. **28**: 143–145.

EGGERT, R., L. T. KARDOS, and R. D. SMITH. 1952. The relative absorption of phosphorus by apple trees and fruits from foliar sprays, and from soil applications of fertilizer, using radioactive phosphorus as a tracer. Proc. Am. Soc. Hort. Sci. **60**: 75–86.

ENDICOTT, K. M., and H. YAGODA. 1947. Microscopic historadiographic technic for locating and quantitating radioactive elements in tissues. Proc. Soc. Exp. Biol. & Med. **64**: 170–172.

EVANS, T. C. 1947. Radioautographs in which the tissue is mounted directly on the photographic paper. Proc. Soc. Exp. Biol. & Med. **64**: 313–315.

FREDRIKSSON, LARS, and LAMBERT WIKLANDER. 1950. [Studies on the uptake of phosphorus by potatoes by means of radiophosphorus]. Kgl. Lantbruksakad. Tid. **89**: 446–459. [Chem. Abs. **45**: 7647-a. 1951].

FREEDMAN, A. J., and DAVID N. HUME. 1950. A precision method of counting radioactive liquid samples. Science **112**: 461–463.

FRENCH, C. B. 1948. Effect of radioactive materials on stimulating plant growth. Canad. Chem. & Proc. Ind. **32**: 225–226.

FRIED, MAURICE. 1948. The absorption of sulphur dioxide by plants as shown by the use of radioactive sulphur. Proc. Soil Sci. Soc. Am. **13**: 135–138.

FULLER, W. H., and L. A. DEAN. 1949. Utilization of phosphorus from green manures. Soil Sci. **68**: 197–202.

GAGER, C. S. 1907. Some effects of radioactivity on plants. Science **25**: 264.

GEIGER, H., and W. MUELLER. 1928. Das Elektronenzählrohr. Physik. Zeits. **29**: 839–841.

———— and ————. 1929. Technische Bemerkungen zum Elektronenzählrohr. Physik. Zeits. **30**: 489–493.

GOMBERG, H. J. 1951. A new high resolution system of autoradiography. Nucleonics **9**: 28–43.

GROSSE, A. V., W. M. JOHNSTON, R. L. WOLFGANG, and W. F. LIBBY. 1951. Tritium in nature. Science **113**: 1–2.

———— and J. C. SNYDER. 1947. Radiographs with $C^{14}$. Science **105**: 240–241.

GUSTAFSON, F. G. 1939. Upward transport of minerals through the phloem of stems. Science **90**: 306–307.

———— and MARJORIE DARKEN. 1937a. Upward transport of minerals through the phloem of stems. Science **85**: 482–483.

———— and ————. 1937b. Further evidence for the upward transport of minerals through the phloem of stems. Am. Jour. Bot. **24**: 615–621

HAHN, L., and G. HEVESY. 1940. Turnover rate of nucleic acid. Nature **145**: 549–550.

HALL, N. S., and A. J. MACKENZIE. 1947. Measurement of radioactive phosphorus. Proc. Soil Sci. Soc. Am. **12**: 101–106.

————, W. L. NELSON, B. A. KRANTZ, C. D. WELCH, and L. A. DEAN. 1949. Utilization of phosphorus from various fertilizer materials. II. Cotton and corn in North Carolina. Soil Sci. **68**: 151–156.

HANSON, J. B., and O. BIDDULPH. 1953. The diurnal variation in the translocation of minerals across bean roots. Plant Physiol. **28**: 356–370.

HARRISON, B. F., M. D. THOMAS, and G. R. HILL. 1944. Radioautographs showing the distribution of sulfur in wheat. Plant Physiol. **19**: 245–257.

HENDERSON, W. J., and U. S. JONES. 1941. The use of radioactive elements for soil and fertilizer studies. Soil Sci. **51**: 283–288.

HENDRICKS, R. H., L. C. BRYNER, M. D. THOMAS, and J. O. IVIE. 1943. Measurement of the activity of radiosulfur in barium sulfate. Jour. Phys. Chem. **47**: 469–473.

HENDRICKS, S. B. 1953. A conference on the use of isotopes in plant and animal research. Kan. Agr. Exp. Sta., Rep. 4: 41–46. [U.S.A.E.C. TID 5098. 1953.]

———— and L. A. DEAN. 1947. Basic concepts of soil and fertilizer studies with radioactive phosphorus. Proc. Soil Sci. Soc. Am. **12**: 98–100.
HENRIQUES, F. C., and C. MARGENETTI. 1946. Analytical procedure for measurement of radioactive hydrogen (tritium). Ind. Eng. & Chem., Anal. Ed. **18**: 420–422.
HEVESY, G. 1923. The absorption and translocation of lead by plants. Biochem. Jour. **17**: 439–445.
————. 1947a. Some applications of radioactive indicators in turnover studies. Adv. Enzm. **7**: 111–214.
————. 1947b. Interaction between the phosphorus atoms of the wheat seedling and the nutrient solution. Ark. Bot., K. Svenska Vetenskapsakad. **33A**: 1–16.
————. 1948a. Historical sketch of the biological application of tracer elements. Cold Spring Harbor Symp. Quant. Biol. **13**: 129–150.
————. 1948b. Radioactive indicators; their application in biochemistry, animal physiology, and pathology.
————, K. LINDERSTROM-LANG, A. S. KESTON, and C. OLSEN. 1940. Exchange of nitrogen atoms in the leaves of the sunflower. Compt. Rend. Lab. Carlsberg, Sér. Chim. **23**: 213–218.
————, ————, and N. NIELSON. 1937. Phosphorus exchange in yeast. Nature **140**: 725.
————, ————, and C. OLSEN. 1936. Atomic dynamics of plant growth. Nature **137**: 66–67.
————, ————, and ————. 1937. Exchange of phosphorus atoms in plants and seeds. Nature **139**: 149–150.
———— and L. ZECHMEISTER. 1920. Über den intermolekularen Platzwechsel gleichartiger Atome. Ber. Deut. Chem. Ges. **53**: 410–415.
HOAGLAND, D. R. 1940. Salt accumulation by plant cells, with special reference to metabolism and experiments on barley roots. Cold Spring Harbor Symp. Quant. Biol. **8**: 181–194.
———— and T. C. BROYER. 1942. Accumulation of salt and permeability in plant cells. Jour. Gen. Physiol. **25**: 865–880.
HOLM-JENSEN, I. B., AUGUST KROUGH, and VEIJO WARTIOVAARA. 1944. Some experiments on the exchange of potassium and sodium between single cells of *Characeae* and the bathing fluid. Acta Bot. Fenn. **36**: 1–22.
HOLT, M. W., R. F. COWING, and SHIELDS WARREN. 1949. Preparation of radio-autographs of tissues without loss of water-soluble $P^{32}$. Science **110**: 328–329.
HOWARD, A., and S. P. PELC. 1951. Synthesis of nucleoprotein in bean root cells. Nature **167**: 599–600.
HUNTER, A. S., and O. J. KELLEY. 1946. A new technique for studying the absorption of moisture and nutrients from soil by plant roots. Soil Sci. **62**: 441–450.
JACOB, W. C., C. H. VAN MIDDELEM, W. L. NELSON, C. D. WELCH, and N. S. HALL. 1949. Utilization of phosphorus by potatoes. Soil Sci. **68**: 113–120.
JACOBSON, H. G. M. 1948. A technique for introducing radioactive phosphorus into the grain of growing corn. Plant Physiol. **23**: 636–637.
JACOBSON, L., and R. OVERSTREET. 1947. A study of the mechanism of ion absorption by plant roots using radioactive elements. Am. Jour. Bot. **34**: 415–420.
———— and ————. 1948. The uptake by plants of plutonium and some products of nuclear fission adsorbed on soil colloids. Soil Sci. **65**: 129–134.
————, ————, H. M. KING, and R. HANDLEY. 1950. A study of potassium absorption by barley roots. Plant Physiol. **25**: 639–647.
JACQUES, A. G. 1940. Separation of potassium isotopes in *Valonia* and *Nitella*. Jour. Gen. Physiol. **23**: 741–742.

JENNY, H., and R. OVERSTREET. 1939a. Surface migration of ions and contact exchange. Jour. Phys. Chem. **43**: 1185–1196.

———— and ————. 1939b. Cation interchange between plant roots and soil colloids. Soil Sci. **47**: 257–272.

————, ———— and A. D. AYERS. 1939. Contact depletion of barley roots as revealed by radioactive indicators. Soil Sci. **48**: 9–24.

KAMEN, M. D. 1946. Survey of contemporary knowledge of biochemistry. I. Isotopic phenomena in biochemistry. Bull. Am. Mus. Nat. Hist. **87**: 105–136.

————. 1951. Radioactive tracers in biology. 2d ed.

KELLY, SALLY, and NORMAN A. BAILE. 1951. The uptake of radioactive iodine by *Ascophyllum*. Biol. Bull **100**: 188–190.

KLECHKOVSKII, V. M., D. D. IVANENKO, V. B. BAGA'EX, and V. V. RACHINSKII. 1947. [The distribution of phosphorus in the plant organs in experiments with radioactive phosphorus]. Doklady Akad. Nauk. S.S.S.R. **58**: 93–95. [Chem. Abs. **45**: 4310-g. 1951].

————, V. N. STOLETOR, and T. P. EVDOKIMOVA. 1951. [Metabolism of labeled phosphorus in grafted plants]. Izvest. Akad. Nauk. S.S.S.R. Ser. Biol. No. 3, 73–85. [Chem. Abs. **46**: 4614-e. 1952].

————, S. P. TSELISHCHER, and T. P. EVDOKIMOVA. 1951. [Determination of labeled phosphorus in leaves of a live plant]. Izvest. Akad. Nauk. S.S.S.R. Ser. Biol. No. 3, 86–90. [Chem. Abs. **46**: 4614-h. 1952].

KOHLMAN, TRUMAN P. 1949. Measurement techniques of applied radiochemistry. Anal. Chem. **21**: 352–364.

KRAMER, P. J. 1951. Effects of respiration inhibitors on accumulation of radioactive phosphorus by roots of loblolly pine. Plant Physiol. **26**: 30–36.

———— and H. H. WIEBE. 1952. Longitudinal gradients of $P^{32}$ absorption in roots. Plant Physiol. **27**: 661–674.

———— and K. M. WILBUR. 1949. Absorption of radioactive phosphorus by mycorrhizal roots of pine. Science **110**: 8–9.

KRANTZ, B. A., W. L. NELSON, C. D. WELCH, and N. S. HALL. 1949. A comparison of phosphorus utilization by crops. Soil Sci. **68**: 171–177.

KROGH, A. 1946. The active and passive exchanges of inorganic ions through the surfaces of living cells and through living membranes generally. Proc. Roy. Soc. London B **133**: 140–200.

LARK-HOROVITZ, K. 1929. A permeability test with radioactive indicators. Nature **123**: 277.

LEBLOND, C. P., M. B. FERTMAN, I. D. PUPPEL, and G. M. CURTIS. 1946. Radioiodine autography in studies of human goiterous thyroid glands. Arch. Path. **41**: 510.

LEGGIERI, L. 1940. [The effect of radioactive waters and of radon on the growth and production of plants]. Ricerca Sci. **11**: 737–753. [Chem. Abs. **35**: 1445–9. 1941].

LEONARD, C. D., and S. J. TOTH. 1950. Plant studies with radioactive sodium. Agron. Jour. **42**: 469–474.

LIBBY, W. F. 1947. Measurement of radioactive tracers. Ind. Eng. & Chem., Anal. Ed. **19**: 2–6.

LONDON, E. S. 1904. Études sur la valeur physiologique et pathologique de l'émanation du radium. Arch. Elect. Med. & Physiotherap. Cancer **12**: 363.

LOW, A. J. 1951. The use of isotopes in agricultural research. II. Chem. & Ind. **51**: 1124–1128.

MACKIE, R. W., J. M. BLUME, and C. E. HAGEN. 1952. Histological changes induced in barley plants by radiation from $P^{32}$. Am. Jour. Bot. **39**: 229–237.

MACKENZIE, A. J., and L. A. DEAN. 1948. Procedure for measurement of $P^{31}$ and $P^{32}$ in plant material. Anal. Chem. **20**: 559–560.

———— and ————. 1950. Measurement of $P^{32}$ in plant material by the use of briquets. Anal. Chem. **22:** 489–490.

MARTIN, R. P. 1952. Electronic apparatus for *in vivo* assay of plants. Nucleonics **10:** 50–53.

———— and R. S. RUSSELL. 1950. Studies with radioactive tracers in plant nutrition. Part I. Problems in the use of radioactive tracers. Part II. The estimation of radioactive tracers. Jour. Exp. Bot. **1:** 133–157.

MAZIA, DANIEL. 1940. The binding of ions by the cell surface. Cold Spring Harbor Symp. Quant. Biol. **8:** 195–203.

———— and L. J. MULLINS. 1941. Radioactive copper and the mechanism of oligodynamic action. Nature **147:** 642.

MCAULIFFE, C. 1949. Determination of radiophosphorus in plant material by solution counting. Anal. Chem. **21:** 1059–1061.

————, N. S. HALL, L. A. DEAN, and S. B. HENDRICKS. 1947. Exchange reactions between phosphates and soils: Hydroxylic surfaces of soil minerals. Proc. Soil Sci. Soc. Am. **12:** 119–123.

———— and M. PEECH. 1949. Utilization by plants of phosphorus in farm manure: I. Labelling of phosphorus in sheep manure with $P^{32}$. Soil Sci. **68:** 179–184.

————, ————, and R. BRADFIELD. 1949. Utilization by plants of phosphorus in farm manure: II. Availability to plants of organic and inorganic forms of phosphorus in sheep manure. Soil Sci. **68:** 185–195.

MILLIKAN, C. R. 1951. Radioautographs of manganese in plants. Austral. Jour. Sci. Res. **B4:** 28–41. [Chem. Abs. **45:** 4787-c. 1951].

MOORE, R. F. 1949. Downward translocation of phosphorus in separated maize roots. Am. Jour. Bot. **36:** 166–169.

MORELAND, D. E. 1950. Translocation of radioactive phosphorus in loblolly pine (*Pinus Taida*). Jour. Elisha Mitchell Sci. Soc. **66:** 175–181.

MUIR, R. M. 1942. Effect of radiation from radioactive isotopes on the protoplasm of *Spirogyra*. Jour. Cell. & Comp. Physiol. **19:** 244–247.

MULLINS, L. J. 1939a. The effect of radiation from radioactive indicators on the penetration of ions into *Nitella*. Jour. Cell. & Comp. Physiol. **14:** 403–405.

————. 1939b. Radioactive isotopes in biology. Phys. Rev. **56:** 1244.

————. 1940. Radioactive ion distribution in protoplasmic granules (of *Nitella coronata*). Proc. Soc. Exp. Biol. & Med. **45:** 856–858.

————. 1941. Ionic equilibria in *Nitella* protoplasm. Jour. Cell. & Comp. Physiol. **18:** 161–172.

————. 1942. The permeability of yeast cells to radiophosphate. Biol. Bull. **83:** 326–333.

————. 1947. The localization of radiophosphate in cells. Proc. Soc. Exp. Biol. & Med. **64:** 296–297.

———— and S. C. BROOKS. 1939. Radioactive ion exchanges in living protoplasm. Science **90:** 256.

———— and KARL ZERAHN. 1948. The distribution of potassium isotopes in biological material. Jour. Biol. Chem. **174:** 107–113.

NAYLOR, J. M., G. W. R. WALKER, D. MURRAY, C. O. PERSON, J. W. T. SPINKS, and T. J. ARNASON. 1951. Uptake of phosphorus$^{32}$ by some cereals. Canad. Jour. Bot. C **29:** 329–338.

NELLER, J. R., and C. L. COMAR. 1947. Factors affecting fixation of phosphorus in soils as determined with radioactive phosphorus. Soil Sci. **54:** 379–387.

NELSON, W. L., B. A. KRANTZ, W. E. COLWELL, W. G. WOLTZ, A. HAWKINS, L. A. DEAN, A. J. MACKENZIE, and E. J. RUBINS. 1947. Application of radioactive tracer technique to studies on phosphatic fertilizer utilization by crops. II. Field experiments. Proc. Soil Sci. Soc. Am. **12:** 113–118.

————, ————, C. D. WELCH, and N. S. HALL. 1949. Utilization of phosphorus as affected by placement: II. In cotton and corn in North Carolina. Soil Sci. **68**: 137–144.

NICKERSON, W. J., and L. J. MULLINS. 1948. Riboflavin enhancement of radioactive phosphate exchange by yeasts. Nature **161**: 939–940.

NISINA, Y., and H. NAKAYAMA. 1938. Absorption and translocation of sodium in the plant. Sci. Pap. Inst. Phys. Chem. Res. [Tokyo] **34**: 1635–1642.

NOGGLE, G. R. 1951. The use of isotopes in soil research. Sci. Mon. **72**: 50–56.

OKUDA, A., Z. KASAI, and Y. ANMA. 1951a. Studies on plant physiology with isotopes. Preliminary report. Distribution of radiophosphorus in various parts of crops. Bull. Res. Inst. Food Sci. Kyoto Univ. No. 5: 34–41. [Chem. Abs. **46**: 6203-c. 1952].

————, ———— and ————. 1951b. I. Absorption and distribution of radioactive phosphorus in various parts of wheat. Bull. Res. Inst. Food Sci., Kyoto Univ. No. 7: 14–18. [Chem. Abs. **46**: 6203-d. 1952].

————, ———— and ————. 1952. Studies on the plant physiology with radioactive isotope. II. Absorption and distribution of phosphorus$^{32}$ in various parts of sesame. Bull. Res. Inst. Food. Sci., Kyoto Univ. No 8: 45–49. 1952. [Chem. Abs. **46**: 7621-g. 1952].

OLSEN, S. R., and R. GARDNER. 1949. Utilization of phosphorus from various fertilizer materials: IV. Sugar beets, wheat, and barley in Colorado. Soil Sci. **68**: 163–169.

OVERSTREET, R., T. C. BROYER, T. L. ISAACS, and C. C. DELWICHE. 1942. Additional studies regarding the cation absorption mechanism of plants in soil. Am. Jour. Bot. **29**: 227–231.

————, and L. JACKSON. 1946. The absorption by roots of rubidium and phosphate ions at extremely small concentrations as revealed by experiments with $Rb^{86}$ and $P^{32}$ prepared without inert carrier. Am. Jour. Bot. **33**: 107–112.

————, and H. JENNY. 1940. Studies pertaining to the cation absorption mechanism of plants in soil. Proc. Soil Sci. Soc. Am. **4**: 125–130.

PACE, NELLO, ROBERT LOEVINGER, and ENRIQUE STRAJNIAN. 1948. *In vivo* Geiger-Müller gamma-ray counter for radioisotope distribution studies. Science **107**: 71–73.

PELC, S. R. 1947. Autoradiograph technique. Nature **160**: 749.

RABIDEAU, G. S., and G. O. BURR. 1945. The use of the $C^{13}$ isotope as a tracer for transport studies in plants. Am. Jour. Bot. **32**: 349–356.

————, W. GORDON WHALEY, and CHARLES S. HEIMSCH. 1948. Relationships between radioactive phosphorus absorption and the developmental pattern of inbred and hybrid maize. Am. Jour. Bot. **35**: 796.

————, ———— and ————. 1950. The absorption and distribution of radioactive phosphorus in two maize inbreds and their hybrids. Am. Jour. Bot. **37**: 93–99.

RABINOWITCH, E. A. 1945. Photosynthesis and related processes. Vol. 1: 10.

RADIN, N. S. 1947, 1948. Isotopic techniques in biochemistry (in 5 parts). Nucleonics **1**(1): 24–33; (2): 48–59; (4): 51–59; **2**(1): 50–56; (2): 33–45.

REDISKE, J. H., and O. BIDDULPH. 1953. The absorption and translocation of iron. Plant Physiol. **28**: 576–593.

REITZ, O., and K. F. BONHOEFFER. 1934. Über den Einbau von schwerem Wasserstoff in wachsende Organismen. Naturwissen. **22**: 744.

ROBINSON, C. V. 1951. A methane, proportional counting method for the assay of tritium. Rev. Sci. Inst. **22**: 353–355.

RUBEN, S., M. RANDALL, M. KAMEN, and J. L. HYDE. 1941. Heavy oxygen ($O^{18}$) as a tracer in the study of photosynthesis. Jour. Am. Chem. Soc. **63**: 877–879.

RUSSELL, R. SCOTT, and R. P. MARTIN. 1949. Use of radioactive phosphorus in plant nutritional studies. Nature **163**: 71–72.

————, F. K. SANDERS, and O. N. BISHOP. 1949. Preparation of radioautographs to show the distribution of phosphorus$^{32}$ in plant tissues. Nature **163**: 639–640.

RUTHERFORD, E. 1899. Uranium radiation and the electrical conduction produced by it. Phil. Mag. **47**: 109–163.

SAYRE, J. D. 1952. Accumulation of radioisotopes in corn leaves. Ohio Agr. Exp. Sta., Res. Bull. 723.

SCHWEITZER, G. K. 1949. The measurement of radioactivity in solution. Jour. Tenn. Acad. Sci. **24**: 123–125.

SIDERIS, C. P. 1950. Manganese interference in the absorption and translocation of radioactive iron ($Fe^{59}$) in *Ananas comosus*. Plant Physiol. **25**: 307–321.

SILBERSTEIN, O., and S. H. WITTWER. 1951. Foliar application of phosphatic nutrients to certain vegetable crops. Proc. Am. Soc. Hort. Sci. **58**: 179–190.

SIRI, WILLIAM E. 1949. Isotopic tracers and nuclear radiations with applications to biology and medicine.

SISAKYAN, N. M., and V. YA. VORONKOVA. 1950. [Radioactive phosphorus exchange between the grafted part and the stock of a hybrid plant]. Doklady Akad. Nauk. S.S.S.R. **70**: 275–277.

SMITH, J. H. C., and DEAN B. COWIE. 1941. Measurement of artificial radioactivity in liquid tracer samples using $C^{11}$. Jour. App. Phys. **12**: 78–82.

SPIEGELMAN, S., and J. M. REINER. 1942. A kenetic analysis of potassium accumulation and soduim exclusion. Growth **6**: 367–389.

SPINKS, J. W. T., and S. A. BARBER. 1946. Study of fertilizer uptake using $P^{32}$. Jour. Am. Chem. Soc. **68**: 2748–2749.

———— and ————. 1947. Study of fertilizer uptake using radioactive phosphorus. Sci. Agr. **27**: 145–156.

———— and ————. 1948. Study of fertilizer uptake using radioactive phosphorus. II. Sci. Agr. **28**: 79–87.

————, E. CUMMINGS, R. L. B. IRWIN, and T. J. ARNASON. 1948. Lethal effects of absorbed radioisotopes on plants. Canad. Jour. Res. C **26**: 249–262.

———— and G. DION. 1949. Study of fertilizer uptake using radiophosphorus. Jour. Chem. Soc., Suppl. **2**: 410–415.

————, ————, M. READE, and J. E. DEHM. 1948. Study of fertilizer uptake using radiophosphorus. III. Sci. Agr. **28**: 309–314.

STANFORD, G., and L. B. NELSON. 1949a. Utilization of phosphorus as affected by placement: I. Corn in Iowa. Soil Sci. **68**: 129–135.

———— and ————. 1949b. Utilization of phosphorus from various fertilizer materials: III. Oats and alfalfa in Iowa. Soil Sci. **68**: 157–161.

STANTON, W. R., and W. K. SINCLAIR. 1951. Effect of high concentration of phosphorus$^{32}$ on growth of the potato. Nature **167**: 234–235.

STOUT, P. R., and D. R. HOAGLAND. 1939. Upward and lateral movement of salt in certain plants as indicated by radioactive isotopes of potassium, sodium, and phosphorus absorbed by roots. Am. Jour. Bot. **26**: 320–324.

———— and W. R. MEAGHER. 1948. Studies of the molybdenum nutrition of plants with radioactive molybdenum. Science **108**: 471–473.

————, R. OVERSTREET, L. JACOBSON, and A. ULRICH. 1947. The use of radioactive tracers in plant nutrition studies. Proc. Soil Sci. Soc. Am. **12**: 91–97.

SWARTOUT, J. A., and M. DOLE. 1939. The protium-deuterium ratio and the atomic weight of hydrogen. Jour. Am. Chem. Soc. **61**: 2025–2029.

THOMAS, M. D., R. H. HENDRICKS, L. C. BRYNER, and G. R. HILL. 1944. A study of the sulphur metabolism of wheat, barley, and corn using radioactive sulphur. Plant Physiol. **19**: 227–244.

————, ————, and G. R. HILL. 1950. Sulfur metabolism in alfalfa. Soil Sci. **70**: 19–26.

TOBIAS, C. A., and R. W. DUNN. 1949. Analysis of micro-composition of biological tissue by means of induced radioactivity. Science **109**: 109–113.

TSAO, TSUNG-HSUN, G. S. RABIDEAU, and W. G. WHALEY. 1950a. The phosphorus uptake of *Andropogon Ischaemum* L. at various stages of development. Plant Physiol. **25**: 653–665.

————, ———— and ————. 1950b. Effects of differences in phosphorus nutrition on the nitrogen content and the phosphorus[32] absorption of four species of grass. Bot. Gaz. **112**: 214–221.

————, and W. G. WHALEY. 1950. Uptake and accumulation of phosphorus[32] by normal and gall tissues. Bull. Torrey Bot. Club **77**: 382–384.

TUCK, J. L. 1939. Radioactive sulphur for biochemical experiments. Jour. Chem. Soc.: 1292–1294.

TURRELL, F. M. 1950. Physiological effects of elemental sulfur dust on citrus fruits. Plant Physiol. **25**: 13–62.

———— and M. CHERVENAK. 1949. Metabolism of radioactive elemental sulfur applied to lemons as an insecticide. Bot. Gaz. **111**: 109–122.

ULRICH, A., L. JACOBSON, and R. OVERSTREET. 1947. Use of radioactive phosphorus in a study of the availability of phosphorus to grape vines under field conditions. Soil Sci. **64**: 17–28.

VINOGRADOV, A. P., and R. V. TEIS. 1941. [Isotopic composition of oxygen of different origins]. Compt. Rend. Acad. Sci. U.R.S.S. **33**: 490–493. [Chem. Abs. **37**: 4094-2. 1943].

VLAMIS, J., and G. A. PEARSON. 1950. Absorption of radioactive zirconium and niobium by plant roots from soils and its theoretical significance. Science **111**: 112–113.

WALKE, H., P. C. THOMPSON, and J. HOLT. 1940. The radioactive isotopes of calcium and their stability as indicators in biological investigations. Phys. Rev. **57**: 177–184.

WEINTRAUB, R. L. 1944. Radiation and plant respiration. Bot. Rev. **10**: 384–459.

WECKMAN, FRANCIS E. 1952. Variations in the relative abundance of the carbon isotopes in plants. Geochim et Cosmochim. Acta **2**: 243–254.

WHITE, J. L., MAURICE FRIED, and A. J. OHLROGGE. 1949. A study of the utilization of phosphorus in green manure crops by the succeeding crop, using radioactive phosphorus. Jour. Am. Soc. Agron. **41**: 174–175.

WILLIAMS, D. E., and N. T. COLEMAN. 1950. Cation exchange properties of plant root surfaces. Plant & Soil **2**: 243–256.

WITHNER, CARL L. 1949. Movement of $P^{32}$ in maturing corn plants. Plant Physiol. **24**: 527–529.

WITTWER, S. H., and W. S. LUNDAHL. 1951. Autoradiography as an aid in determining the gross absorption and utilization of foliar applied nutrients. Plant Physiol. **26**: 792–797.

WOLFGANG, R. L., and W. F. LIBBY. 1952. Absolute excitation function of the $Be^{9}$ (d, t) reaction. Phys. Rev. **85**: 437–440.

WOLTZ, W. G., N. S. HALL, and W. E. COLWELL. 1949. Utilization of phosphorus by tobacco. Soil Sci. **68**: 121–128.

YANKWICH, PETER E. 1949. Radioactive isotopes as tracers. Anal. Chem. **21**: 318–321.

YARWOOD, C. E., and LOUIS JACOBSON. 1950. Selective absorption of sulfur[35] by fungus-infected leaves. Nature **165**: 973–974.

Future Contents of

# THE BOTANICAL REVIEW

## Articles Received and Awaiting Publication

| | |
|---|---|
| **Fungi That Attack Microscopic Animals** ..... | C. L. Duddington<br>*The Polytechnic, London* |
| **The Phytogeography of Unglaciated Eastern United States and Its Interpretation** ..... | E. Lucy Braun<br>*Cincinnati, Ohio* |
| **Genetics and Selective Breeding of Sugar Beets in the USSR** ..... | Alexander Archimowitsch<br>*Agricultural Institutes in USSR* |
| **Control of Plant Diseases by the Use of Antibiotic Organisms** ..... | R. K. S. Wood and M. Tveit<br>*Imperial College, London* |
| **The Vegetation of Alberta** ..... ..... | R. H. Moss<br>*University of Alberta* |
| **Dwarf Mistletoes** ..... | Jos Kuijt<br>*Canada Dept. of Agriculture* |
| **Prospects of Application of Biological Control in Forest Pathology** ..... | Mihailo Krstic<br>*Belgrade, Yugoslavia* |
| **The Medullosan Pteridosperms** ..... | Wilson N. Stewart<br>*University of Illinois*<br>and<br>Theodore Delevoryas<br>*University of Michigan* |
| **Ecological Life History Outlines for Algae** .... | G. W. Prescott<br>*Michigan State College* |
| **Cytogenetics of the Vegetable Crops. II. Crucifers** ..... | S. H. Yarnell<br>*U. S. Department of Agriculture* |
| **The Ecology of River Algae** ..... | John L. Blum<br>*Canisius College* |
| **Drought Resistance in Woody Plants** ..... | Johnson Parker<br>*University of Idaho* |
| **Fine Structure in Degraded, Ancient and Buried Wood, and Other Fossilized Plant Derivatives** ..... | J. Sen<br>*Calcutta University* |
| **The Plant Centrosome and the Centrosome-Blepharoplast Homology** ..... | Robert Lepper<br>*University of Rhode Island* |
| **Recent Trends in Palynology** ..... | Knut Faegri<br>*Universitetet, Bergen, Norway* |
| **Chromosomal Interchanges in Plants** ..... | C. R. Burnham<br>*University of Minnesota* |